SOLVATOCHROMIC SOLVENT POLARITY MEASUREMENTS,
RETENTION, AND SELECTIVITY
IN REVERSED PHASE LIQUID CHROMATOGRAPHY

BY

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This dissertation is dedicated
to my son, Garrett Chase, whose
arrival coincided with the
completion of this work.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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The $E_T(30)$ polarity and π^* dipolarity/polarizability
of binary acetonitrile/water and methanol/water mobile
phases used in reversed-phase liquid chromatography were
measured and compared with chromatographic retention and
selectivity. For the retention data, plots of log k'
versus the $E_T(30)$ polarity were generally found to be
better descriptors of retention than the more commonly used
plots of log k' versus percent organic modifier. A total
of 332 sets of retention data were examined, and the
overall average r^2 values obtained for simple linear
regression of log k' versus either percent organic modifier
or $E_T(30)$ values were 0.9783 and 0.9910, respectively.

The slope and y-intercepts of plots of log k' versus
$E_T(30)$ polarity were found to be dependent on the solute

size, solvent system, and the column. Also, for a given column and solute, the slope for the two solvent systems examined was found to vary in systematic matter, with that of methanol/water mixtures 1.43 times greater (on the average) than that for acetonitrile/water mixtures. This variation in slope is evidence of differential solvation of the bonded phase alkyl chains by the two organic modifiers.

In addition, the variation in methylene selectivity as a function of either percent organic modifier or $E_T(30)$ polarity has been examined for various bonded phases, as well as for a polymer-based column.

Solvatochromic solvent polarity measurements offer a unique view of the retention process, by providing a means of determining mobile phase polarity that is independent of the chromatographic system, thus allowing de-convolution of subtle stationary phase solvent effects, as well as the prediction of chromatographic retention.

CHAPTER I
INTRODUCTION

The actual mechanism of retention in reversed phase liquid chromatography (RPLC) has been the subject of much controversy and debate since the first bonded phases for chromatography became commercially available in the early 1970s. Despite its name, reversed phase liquid chromatography is actually a more "popular" technique than normal phase liquid chromatography (NPLC). Stationary phases used in RPLC typically consist of a silica-based supporting material to which nonpolar carbon chains are bonded. These carbon chains are most commonly straight chains of length 8 or 18 carbons (hence the terms "octyl," "octadecyl," C-8, C-18, etc. to describe the type of bonded phase). The carbon chains are attached through a bonding reaction in which the surface hydroxyl groups present on the silica (silanols) are reacted with the appropriate chlorosilane, leading to an Si-O-Si-C bond. For example, to produce an octadecyl bonded phase, one could react dimethyloctadecylchlorosilane with silica. The bonding reaction is not exhaustive, however, so in a second step trimethylchlorosilane or hexamethyldisilazine is typically added in order to "endcap" the residual silanols that may

not be accessible to the larger, more sterically hindered silane used in the first bonding reaction.

An ideal bonded phase would have no residual silanols and would possess univariate pore and particle size distributions. Practically speaking, no bonded phase can be said to be free of residual silanol groups; one of the major differences between competing commercial stationary phases is in the degree of endcapping.

The presence of residual silanols is highly undesirable since it leads to a second mechanism of retention. Polar compounds and/or ones possessing hydrogen bond donor/acceptor ability can interact with these silanols (silanophilic solutes), leading to distorted peak shape and/or greatly increased retention (Bij et al., 1981; Nahum and Horvath, 1981). This problem was recently reviewed, and the effects of both silanophilic and metallophilic interactions were compared (Sadek et al., 1985a). It was found that stainless steel inlet frits commonly employed in LC columns also cause losses in efficiency, due to both mechanical and chemical interactions. Silanophilic interactions were found to be the major factor in affecting the retention of basic amine compounds.

While the mechanism of normal phase liquid chromatography (NPLC) can be said to be fairly well characterized in terms of adsorption at active sites upon

the silica or alumina surface, that of RPLC remains controversial. In general, one can distinguish two broad areas of study of this mechanism. These two areas are referred to as "mobile phase effects" and "stationary phase effects."

Mobile Phase Effects

In the first of these ("mobile phase effects"), one observes or calculates the effects of changing mobile phase composition on chromatographic retention. Typical mobile phases used in RPLC consist of water to which an organic modifier has been added. The most frequently used organic modifiers are methanol, acetonitrile, and tetrahydrofuran.

One example of the approaches classified as "mobile phase effects" is that of solubility parameter theory. Hildebrand's solubility parameter has been shown to be useful in the prediction of many solution properties and is defined by

$$\delta = (E/V)^{1/2} \qquad (1\text{-}1)$$

where E is the molar heat of vaporization of the solvent and V is its molar volume. When applied to chromatography, retention is viewed in terms of the relative solubility parameters of the solute, mobile phase, and the stationary phase (Karger et al., 1978; Schoenmakers et al., 1982).

Capacity factors can then be considered to be related to these parameters, as shown in the following equation:

$$\ln k' = (v/RT)(\delta_m + \delta_s - 2\delta_i)(\delta_m - \delta_s)$$

$$+ \ln (n_s/n_m) \qquad (1-2)$$

where v is the molar volume of the solute and δ_m, δ_s, and δ_i are the solubility parameters of the mobile phase, stationary phase, and solute, respectively. The (n_s/n_m) term is the ratio of moles of the stationary and mobile phases, respectively. If the solubility parameter for a solvent mixture is approximated by assuming linear additivity of volume fractions, the dependence of retention on the volume fraction of one of the components becomes

$$\ln k' = A (\phi)^2 + B (\phi) + C \qquad (1-3)$$

where A, B, and C are constants. Assuming linear additivity of solubility parameters is questionable, however. Particularly for aqueous mixtures, where hydrogen bond forces have a very large effect on the heat of vaporization, the solubility parameter is likely to be complex function of the volume fraction of the components. This equation also results from assuming that the stationary phase solubility parameter is a constant,

regardless of the mobile phase composition, which is also a questionable assumption (see discussion of stationary phase solvation in the latter part of this section). Moreover, it is impossible to measure the solubility parameter of either the binary/ternary solvent mixtures used in RPLC, or the alkyl chains of the stationary phase. While the theory enables qualitative predictions to be made on the basis of relative polarity of the solute and phases, quantitative calculations are not possible.

Hafkenscheid and Tomlinson (1983) have recently re-cast solubility parameter theory for RPLC. Semi-empirical relationships were derived in order to allow more accurate predictions of retention. Other workers have subdivided the solubility parameter into individual contributions due to dispersive forces, proton transfer, polar interactions, etc., in an attempt to predict retention with greater accuracy. An example of this approach would be the work of Tjissen et al. (1976). Unfortunately, as the accuracy of prediction increases the practicality of applying such complex equations also decreases in an inverse fashion.

One of the most well-known approaches to chromatographic retention is the hydrophobic theory of Sinanoglu (1968), as applied by Horvath and Melander (1977). This model (also referred to as the solvophobic model) describes retention in terms of repellent forces between the relatively nonpolar solute and the highly polar

6

aqueous mobile phase. This results in the formation of a complex between the stationary phase ligands and the relatively hydrophobic solute. Here the stationary phase acts as a passive receptor to hydrophobic molecules that are repelled by the aqueous mobile phase (cavity effect). The stationary phase is treated as a constant, and specific interactions between residual silanols and polar groups on the solute molecules are not treated. Various mathematical expressions were derived relating retention to solute properties such as the hydrocarbonaceous surface area (HSA) and solvent properties such as the dielectric constant or surface tension.

Recently, Antle et al. (1985) compared various RPLC columns with respect to solvophobic selectivity. Retention differences seen among columns were ascribed to three effects: differences in phase ratio, the polarity of the bonded phase, and the dispersion solubility parameter of the stationary phase.

Martire and Boehm (1980, 1983) have applied statistical-mechanical theory to the description of chromatographic retention. Using a lattice model, predictions were made about the effects of either changing mobile phase composition or length of alkyl bonded groups. Though these derivations are quite rigorous, practical application of the results is somewhat

difficult.   Furthermore, because of several assumptions
made, again only qualitative predictions are possible.

Interaction indices (empirical measurement of I values
based on solute retention) have also been used by Jandera
et al. (1982) to predict retention.   Here it is assumed
that the empirical interaction index of a solvent mixture
is a linear sum of the volume fraction contributions of the
solvents   and   again   only   qualitative   predictions   are
possible.

Stationary Phase Effects

In the second broad area of study ("stationary phase
effects"), the nature of the stationary phase is studied
through either direct physical measurement or through the
effects of changing the type of bonded phase (i.e., C-2,
C-8, etc.) on chromatographic retention.   Direct physical
measurements   of   the   stationary   phase   involve   either
spectroscopic methods or actual chemical dissolution.

The most basic chemical analysis of a stationary phase
is the determination of percent carbon.   The percentage of
carbon loading provides information about the extent of the
bonding reaction, as well as the degree of surface coverage
(assuming   the   surface   area   has   been   determined).   Of
course, this provides no information about the conformation
or   spatial   distribution   of   the   alkyl   chains.   Other
dissolution methods have been used in an attempt to examine

the chemical form of the bonded alkyl chains. For example, Lullman et al. (1985) carried out studies in which bonded phase packings were fused with potassium hydroxide. In this manner, the alkyl ligands were cleaved from the silica substrate, and subsequent GC analysis of the fusion products revealed the presence of hexaalkyldisiloxanes and trialkylsilanols for monomeric bonded phases. Another approach is to digest the stationary phase in hydrofluoric acid and then to subject this digest to analysis by gas chromatography (Fazio et al., 1985). Based on GC analysis of these digests, it was possible to distinguish between the various methods used to derivatize the silica (i.e., whether mono-, di-, or tri-chlorosilanes had been used).

While the stationary phase is often treated as a passive or invariant entity, there is much evidence that the solvation of the alkyl ligands themselves changes in response to varying composition of the mobile phase. This is best exemplified by the re-equilibration necessary after an organic concentration gradient. That is, the alkyl chains that comprise the stationary phase surface are preferentially solvated by the organic component of the mobile phase. Because of this, the interfacial region between the bulk mobile phase and the surface of the silica base has widely varying physical properties, so that chromatographic retention reflects the statistical mean of these varying physical properties. The organic modifier

content of the stationary phase increases with the concentration of modifier in the mobile phase (Yonker et al., 1982a, 1982b). Among the three most commonly used organic modifiers, tetrahydrofuran has been shown to solvate the stationary phase to the greatest extent, followed by acetonitrile and methanol (Yonker et al., 1982a, 1982b). Thus, while the mobile phase may consist of a 50/50 mixture, the stationary phase will be solvated by a mixture with a significantly higher proportion of the organic modifier.

Lochmuller and Wilder (1979) compared the selectivity of various bonded phases with that of equivalent liquid-liquid systems. For chain lengths greater than approximately 12 carbons, the selectivity was found to be comparable to the liquid-liquid system. Also, Lochmuller et al. (1981) prepared bonded phases with either n-heptyl, cyclohexyl, or bicycloheptyl alkyl chains. The n-heptyl phase was found to have the highest selectivity and capacity, though the cyclic phases were found to retain cycloalkanes preferentially. Jinno and Okamato (1984) prepared bonded phases with various aromatic moieties. Capacity factors were measured for various polynuclear aromatic hydrocarbons (PAHs). In this case, the pore size of the silica matrix appeared to influence retention, either because of its effect on the bonding reaction or

varying abilities of the PAHs to penetrate the interior pores (steric effects).

One way to use a spectroscopic method in characterizing the stationary phase is to sorb or chemically bond a probe molecule to the surface and then observe the electronic spectrum of the probe molecule. Fluorescence spectroscopy lends itself to this type of measurement, because of the type of sample and the inherent lower detection limits possible. Since the sample is a solid, it is very difficult to observe the adsorbed species directly through absorption spectroscopy. That is, the solid silica particles tend to scatter the incoming light beam to a greater degree; using a lower amount of suspended solid lowers the degree of light scattering at the expense of lowered sensitivity to the presence of the probe molecule. A secondary problem is the need to apply very small amounts of the probe molecule to the stationary phase. If too much is applied, more than a monolayer may be formed, and thus the resultant information is of questionable value. Also, one would not want to "overload" the packing, i.e., operate at a concentration where the sorption isotherm becomes nonlinear, which would also yield results not applicable to the true conditions seen by the stationary phase. For these reasons, fluorescence (for UV/VIS) or diffuse reflectance (for infrared or UV/VIS) spectroscopy are ideally suited to this type of

experiment. Of course, it is essential that for fluorescence experiments, the excitation and emission wavelengths be sufficiently separated to avoid interference from the aforementioned scattered light.

In choosing a probe molecule to study the stationary phase, the two most important criteria are spectral response to changing solvent polarity and affinity for the bonded alkyl chains. A probe molecule with insufficient affinity (i.e., too low of a partitioning coefficient between the mobile and stationary phases) will reside in the mobile phase to such an extent that the fluorescence cannot be attributed solely to that residing on the stationary phase. For these reasons, the most commonly used probe molecule has been pyrene, a 4-ring fused aromatic compound. The fluorescence spectrum has vibronic structure which is quite sensitive to the solvent environment. In fact, this molecule has been used to establish the Py scale of solvent polarity (Dong and Winnik, 1984; empirical solvent polarity scales are discussed in detail in the latter part of this chapter). The fluorescence spectrum of pyrene contains five major vibronic bands, labeled I to V, beginning with the 0-0 band. The ratio of the intensities of bands I and III has been shown to be highly responsive to changing solvent environment. Being a large, hydrophobic molecule, it has a very large affinity for the alkyl chains of the stationary phase. Two recent

12

papers have reported on the variation in stationary phase polarity (as seen by pyrene sorbed onto the column packing) as a function of the mobile phase composition (Carr and Harris, 1986; Stahlberg and Almgren, 1985).

It is interesting to note that these two groups obtained data for complementary organic modifier concentration ranges, as a result of the experimental conditions used. Stahlberg and Almgren (1985) measured the surface polarity of C-2 and C-18 surfaces in the presence of 0-30% methanol/water and acetonitrile/water mixtures. This was done by using a suspension of 2-8 mg packing per mL of solvent. Sodium tetradecylsulfate was also added (0.5 mg/mL) to prevent flocculation of the particles. In the 0-30% acetonitrile range, the surface polarity of the C-18 packing was found to be greatest at the extremes, while in methanol it decreased steadily as the concentration increased. At higher concentrations of organic modifier (>30% v/v), the concentration of pyrene in the solvent mixture became too great and thus obscured the fluorescence spectrum of the sorbed material. The interpretation of these results is complicated, however, because of the presence of added surfactant (0.5 mg/mL; 0.0015 M), which is also likely to sorb onto the bonded chains and modify the surface polarity. Carr and Harris (1986) studied both polymeric and monomeric C-18 phases in a similar manner, except that the sample consisted of a

flow-cell packed with the solid, through which the solvent mixture with pyrene was passed. In this case, the investigators were limited to concentrations greater than 20, 25, and 50% acetonitrile, tetrahydrofuran, and methanol, respectively, because the entire packed particle bed could not be fully equilibrated with pyrene. Here the ratio of stationary phase to mobile phase volume was much higher, leading to a much greater quantity of sorbed pyrene.

This effect can be demonstrated quantitatively by the following equation relating capacity factor (k') to the thermodynamic distribution coefficient (K) and phase ratio (ϕ):

$$k' = k\phi \qquad (1-4)$$

The phase ratio, ϕ, is the ratio of the volumes of the stationary and mobile phases. The capacity factor, k', corresponds to the ratio of the moles of the sorbed material present in the stationary and mobile phases at any given instant. Thus, the packed bed used by Carr and Harris (1986) has a much higher value than the slurry used by Stahlberg and Almgren (1985), making the use of higher organic modifier concentrations necessary (lower K values). On the other hand, the upper limit of organic modifier concentration is increased, since the higher value

compensates for the greatly decreased affinity of pyrene for the stationary phase (lower K). Thus, Carr and Harris (1986) were able to report surface polarities for up to 80, 45, and 70% methanol, tetrahydrofuran, or acetonitrile, respectively. For a C-18 monomeric packing, the surface polarity was found to increase with increasing organic modifier concentration, with methanol systems having consistently lower polarity than that of the acetonitrile or tetrahydrofuran systems. This is a direct reflection of the fact that much less methanol is absorbed by the alkyl chains as the organic concentration is increased, so the polarity remains closer to that of a pure alkane. On the other hand, much greater amounts of acetonitrile and tetrahydrofuran solvate these alkyl chains, leading to an increase in apparent polarity (with respect to a pure alkane). These results are fully consistent with those of earlier workers who measured the adsorption isotherms of organic modifiers onto various column packings. For example, McCormick and Karger (1980a, 1980b) and Tanaka et al. (1980) reported the organic modifier content of reversed phase column packings under various concentrations. Even at a concentration of 10% organic modifier, acetonitrile was found to solvate the alkyl chains to a much higher degree than methanol.

One way to get around the problem of lowered affinity of the probe for the stationary phase at high organic

modifier concentration is to simply immobilize it by bonding it to the stationary phase. Lochmuller et al. (1985) measured the fluorescence of surface bonded exciplexes (pyrene/N,N-dimethylaniline) to measure the surface polarity after endcapping with either trimethylchlorosilane (TMCS) or hexamethyldisilazine (HMDS). Trimethylchlorosilane was found to yield a stationary phase of lower polarity.

Another major area of spectroscopic examination of stationary phases involves the use of nuclear magnetic resonance (NMR). As with the sorbed probe fluorescence experiments, the greatest wealth of information is derived from those in which the packing is examined under "real" conditions, i.e., in the presence of a mobile phase. Most often, ^{13}C is used in NMR experiments. One problem that must be overcome is the low signal to noise ratio of ^{13}C-NMR, which is aggravated by the nature of the sample. Also, alkyl chain C-atoms have nearly identical chemical shifts, making it difficult to differentiate between individual positions within the chain. Gilpin and Gangoda (1984, 1985) have synthesized stationary phases in which the terminal carbon atom is enriched with the ^{13}C isotope, thus overcoming some of these difficulties. The ^{13}C spin-lattice relaxation times (in either pure deuterated chloroform or acetonitrile) were found to be fairly constant for the various chain lengths studied. However,

at higher coverage densities, a decrease was noted. This is evidence for the increasing interaction between the neighboring alkyl chains. The effect of solvent viscosity was also explored; an inverse relationship was found between spin-lattice relaxation time and solvent viscosity. Also, ^{29}Si-NMR with magic angle spinning has been used to differentiate between the various chemical environments of the silicon atoms in dry samples of column packings (Fyfe et al., 1985).

Fourier transform infrared spectroscopy (FTIR) has been used to directly observe the stationary phase alkyl chains. Again, experiments have been done with both dry packings and in the presence of solvent mixtures. In this case, a major difficulty arises from the strong infrared absorption band of water and methanol (O-H stretching), which tends to obscure the C-H stretching band of the bonded alkyl chain. One solution to this problem is to use deuterated solvents, as Sander et al. (1983) have done with C-1 to C-22 column packings. The range of 70-100% methanol was studied, and evidence of increasing chain order was found at the higher organic concentrations. Also, temperature studies were carried out on the dry packings, and no phase transitions were observed at temperatures near or below the corresponding alkanes. The degree of disorder of the chains was found to be comparable to that of liquid n-alkanes at room temperature; thus the surface of the

bonded phase behaves like silica with a thin oily coating. Suffolk and Gilpin (1985) have made FTIR measurements of a cyanoalkyl bonded phase. Here the cyanoalkyl group could easily be observed with little interefrence from the solvent. In hexane, there appeared to be two distinct populations of bonded ligands (possibly due to interaction with surface silanols), while in 1-butanol ligand-solvent interaction was more apparent.

Other studies of the stationary phase have made use of such diverse analytical techniques as differential scanning calorimetry (Hansen and Callis, 1983), ESCA (Miller et al., 1984), and photoacoustic spectroscopy (Lochmuller et al., 1980; Miller et al., 1984). Recently, two general reviews of stationary phase structural studies have been published (Gilpin, 1984, 1985).

Despite the plethora of spectroscopic studies published on the nature of the stationary phase, in no case is a quantitative relationship derived between these experimental results and actual chromatographic retention. In all cases, the spectroscopic results are interpreted in a qualitative manner.

Empirical Measures of Solvent Polarity

For any chemical process occurring in solution, the polarity of the solvent plays a crucial role in determining the outcome. While this has been known for many years,

only recently has the exact role of the solvent begun to be clarified and quantitated. Solvent properties influence not only the rates of chemical reactions, but also the position of chemical equilibria. Many spectral properties are affected by the nature of the solvent. It is well known that both the intensity and absorption or emission frequency of NMR, IR, UV/VIS, and luminescence spectra are affected by the solvent. This is an example of solvatochromism, in which the position, intensity, or shape of a spectral peak is affected by the solvent. The importance of solvatochromism is demonstrated quite clearly by the Sadtler library of standard ultraviolet spectra (Sadtler Research Laboratories, Philadelphia, PA), in which the spectra are reported for solutes dissolved in methanol, wherever solubility permits. In this way, peak positions for different substances are easily compared, with no need to correct for the effect of different solvents.

In the field of analytical chemistry, solvent effects must be taken into account when developing a method of analysis. Solvents will affect the position and intensity of spectral peaks being measured in quantitative IR or UV/VIS spectroscopy, the rates and extent of reactions used in derivatization or titration, etc. Also, chemical separations by liquid chromatography (either normal or reversed phase), which are controlled primarily by the nature of the solvent(s) used as the mobile phase, are a

direct result of the different polarities of the stationary and mobile phases.

There are many ways to characterize the polarity of a solvent. Bulk physical properties, such as dielectric constant, viscosity, or refractive index represent the simplest measures of solvent properties. However, no single physical property can adequately characterize the "polarity" of a solvent. The "polarity" of a solvent is extremely difficult to define and represents the sum total of all possible interactions that a solute may experience when dissolved in a particular medium. Therefore, bulk or macroscopic properties will only provide information about the interaction between the solvent molecules themselves. Interactions that a solute may experience include dispersion, dipole-dipole, dipole-induced dipole, and hydrogen-bond forces. Because of the difficulty of characterizing the polarity of a solvent through bulk physical properties, a number of empirical scales of solvent polarity have been developed in the past 50 years. These empirical scales are based on the properties of particular solutes dissolved in the solvent of interest. In this way, specific, microscopic interactions with the solvent are probed, since the test solute is able to "see" these better than bulk, macroscopic properties can. Extensive reviews of empirical measures of solvent

polarity have been published (Griffiths and Pugh, 1979; Reichardt, 1979).

The earliest empirical scales of solvent polarity were based on a kinetic measurement of some reaction carried out in the solvent of interest. Perhaps the most well-known scale of this type is the Y-scale, developed by Grunwald and Winstein (1948). The Y-scale is based on the solvolysis of t-butyl chloride. The rate constant for this first order process is measured, and a Y-value is calculated with the following equation:

$$\log k - \log k_0 = mY \tag{1-5}$$

where k is the rate constant, k_0 is the rate constant in 80% (aqueous) ethanol, and m is the sensitivity of the substrate (m = 1 for t-butyl chloride, by definition).

There are also many empirical scales of solvent polarity based on a spectroscopic measurement. The fact that a solvent will influence the spectral properties of a solute is used as a way of characterizing solvent polarity. These measurements are quite simple and involve nothing more than dissolving the test solute in the solvent and recording the absorption or emission spectrum (either IR, NMR, or UV/VIS). One example of this type of scale is that of Kosower's Z-values (Kosower, 1958), which are based on the intermolecular charge-transfer absorption of

1-methyl-4-carbomethoxypyridinium iodide. The Z-values are
defined by

$$Z = 28592/\lambda_{max} \qquad (1-6)$$

where λ_{max} is the position of the charge transfer peak (in
nm).   The constant in equation 1-6 is the product of
Avogadro's   number,   the   speed   of   light,   and   Planck's
constant. The Z-values have been reported for more than 50
pure solvents and solvent mixtures (Kosower, 1958).   For
mixtures with a high water content, the charge transfer
peak merges with that of the aromatic ring and is unable to
be located.

The π^* scale of solvent polarity was developed by
Kamlet et al. (1977). Its name derives from the fact that
it is based on the positions of the π to π^* transitions of
a series of chromophores. Rather than a single solute, it
is based on a series of aromatic solutes, in which the π^*
parameter   was   adjusted   to   give   the   most   consistent
correlation among the various test solutes.   The inventors
of this scale prefer to refer to it as the π^* scale of
solvent dipolarity/polarizability.   In fact, in solvents
with no potential for hydrogen bonding, there is a linear
correlation between the molecular dipole moment and the
measured π^* value.   Brady and Carr (1982, 1985) have
discussed this scale in terms of the Onsager reaction field

and Block and Walker dielectrically saturable reaction field models. For a given solute, π^* values are calculated with the following equation:

$$\pi^* = (\nu - \nu_0)/s \qquad (1-7)$$

where s is the sensitivity of the solute to the π^* scale, and ν and ν_0 are the absorption maxima (X 10^{-3} cm^{-1}) of the solute in the solvent and cyclohexane, respectively. The appropriate constants for this equation have been published (Kamlet et al., 1977). In addition both α and β measures of solvent hydrogen bond donor and acceptor ability have been derived from these same solutes. These measures are based on the enhanced solvatochromic shift of one indicator relative to another in the presence of hydrogen bond donor/acceptor solvents. For example, one way to measure the β value is to compare the solvatochromism of 4-nitroanisole with respect to 4-nitrophenol; solvents capable of hydrogen bond acceptor interactions will cause an enhanced solvatochromic shift for the 4-nitrophenol with respect to 4-nitroanisole (Kamlet and Taft, 1976). In a similar manner, solvent α values can be derived from the enhanced solvatochromic shift of ET-30 with respect to 4-nitroanisole (vide infra).

The $E_T(30)$ scale of solvent polarity was developed in the early 1960s by Dimroth et al. (1963a, 1963b) and

Reichardt (1979), who reported on the solvatochromism of a series of 42 pyridinium betaine dye molecules. In the original paper, derivative #30 was found to have the greatest sensitivity to changes in solvent polarity. Thus, the $E_T(30)$ scale was named as such because it is derived from the molar energy of transition (E_T) of the thirtieth pyridinium betaine (30). The $E_T(30)$ scale of solvent polarity is based on the intramolecular charge transfer absorption of 2,6-Diphenyl-4-(2,4,6-triphenyl-N-pyridino)-phenolate (structure shown in Figure 1-1). It possesses a number of unique features, such as a 44-electron aromatic ring system, a negatively charged phenoxide group and a positively charged pyridine ring nitrogen atom. This molecule undergoes one of the largest known shifts in λ_{max}, amounting to some 357 nm in going from water (453 nm) to diphenyl ether (810 nm). Since this dye absorbs within the visible light region, it is possible to estimate visually the polarity of a solvent. In methanol, the solution is wine-red, while in acetonitrile the solution becomes deep blue in color. Values of $E_T(30)$ polarity are calculated in the same manner as are Z-values (equation 1-6) and have been reported for over 200 solvents (Reichardt and Harbusch-Gornert, 1983). Also, the range of the scale has been expanded through the use of a more lipophilic betaine, in which a t-butyl group is attached to each of the five
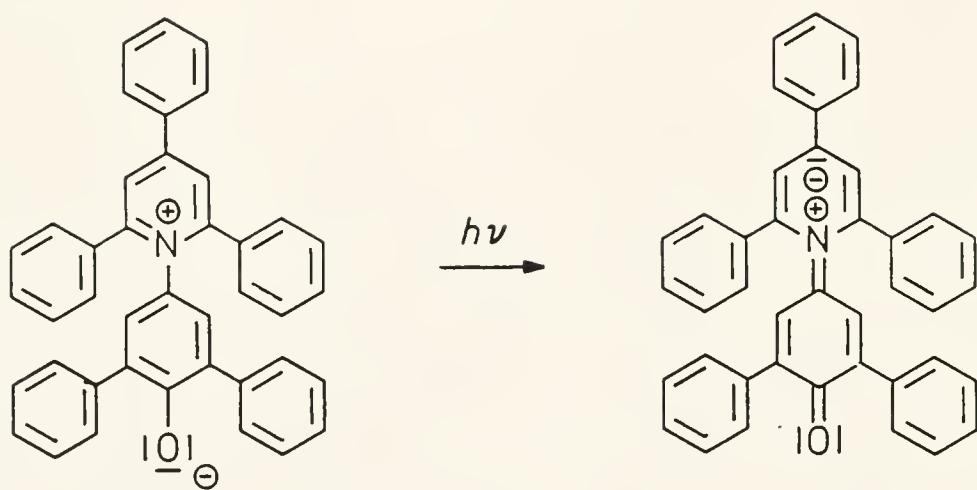
Figure 1-1. Structure of the ET-30 dye molecule, 2,6-Diphenyl-4-(2,4,6-triphenyl-N-pyridino)-phenolate in the ground and excited states.

phenyl groups (para position). Recently, a normalized
scale of $E_T(30)$ polarity (E_T^N) has been defined by Reichardt
and Harbusch-Gornert (1983), in which the polarity of water
is defined to be 1.0, while that of tetramethylsilane (TMS)
is 0. These values are calculated by using the following
equation:

$$E_T^N = \frac{E_T(\text{solvent}) - E_T(\text{TMS})}{E_T(H_2O) - E_T(\text{TMS})} \qquad (1-8)$$

where $E_T(\text{solvent})$ is the $E_T(30)$ polarity of the solvent in
question as calculated by equation 1-6, and $E_T(H_2O)$ and
$E_T(\text{TMS})$ have values of 63.1 and 30.7, respectively. The
normalized scale is used for convenience in expressing a
polarity relative to water or tetramethylsilane and has no
actual effect on the types of correlations discussed
herein. All $E_T(30)$ polarity values reported here are in
kcal/mole, as calculated from equation 1-6.

The $E_T(30)$ scale has been shown to be sensitive to
both solvent dipolarity/polarizability as well as solvent
hydrogen bond donor ability (HBD). Taft and Kamlet (1976)
calculated that 68% of the shift in λ_{max} in going from
cyclohexane to n-butanol is due to HBD stabilization of the
ET-30. This stabilization is a direct result of the
presence of the negatively charged phenoxide group on the
ET-30 molecule. The phenoxide group acts as a hydrogen
bond acceptor, so that protic solvents may function as

suitable H-bond donors. In fact, Taft and Kamlet (1976) have used the enhanced solvatochromic shift of ET-30 with respect to 4-nitroanisole to measure the solvent hydrogen bond donor acidity (α-scale). In both protonic and nonprotonic solvents, the $E_T(30)$ scale can be related to the π^* and α scales by use of the following equation (derived from equation 7 of Kamlet et al., 1976):

$$E_T(30) = 30.31 + 14.6\pi^* + 16.53\alpha \qquad (1-9)$$

Analytical Applications of the ET-30 Dye

Owing to its extreme sensitivity to changes in overall solvent polarity, ET-30 may be used to determine the composition of binary solvent mixtures. However, ET-30 is particularly sensitive to the presence of small amounts of water in aprotic solvents. For protic solvents, the presence of water has a smaller effect, as illustrated with tert-butyl hydroperoxide. Langhals et al. (1980) have reported that the presence of 5.2 moles/liter water changes the apparent solution color from blue (λ_{max} = 575 nm) to red (λ_{max} = 532 nm). Thus, the color of the solution serves as a visual indicator of the water content, and measurement of λ_{max} for ET-30 dissolved in a given solvent can be a rapid and precise alternative to Karl-Fischer water determinations. Of course, quantitation of the water

content for a given solvent requires that the $E_T(30)$ value be known for each composition. Values of $E_T(30)$ polarity for many binary solvent systems have been reported (Balakrishnan and Easteal, 1981a, 1981b; De Vijlder, 1982; Dimroth and Reichardt, 1966; Jouanne et al., 1978; Koppel and Koppel, 1983a, 1983b; Krygowski et al., 1985; Maksimovic et al., 1974). If the variation in ET-30 as a function of composition is monotonic, i.e., no maxima or minima occur, this determination is fairly straightforward. On the other hand, if there are any maxima or minima, this is not possible, since a given λ_{max} value will correspond to more than one concentration. This would be the case, for example, for mixtures of acetonitrile with isopropanol, as reported by Koppel and Koppel (1983a). Langhals (1982a) has proposed the following equation to follow changes in $E_T(30)$ polarity values in binary solvent mixtures:

$$E_T(30) = E_d \ln(C_p/C^* + 1) + E_T^0(30) \qquad (1-10)$$

where C_p is the molar concentration of the most polar component, E_d and C^* are constants determined for each binary system, and $E_T^0(30)$ is the $E_T(30)$ polarity for the least polar solvent. The appropriate constants for a total of 46 binary solvent systems have been reported, as well as for an organic co-polymer (Langhals, 1982a, 1982b). This

28

equation is discussed in further detail in Chapter III. Alternatively, the change in absorbance of a solution of ET-30 at a fixed wavelength has been used to determine mixture composition. For example, Kumoi et al. (1970) have reported that water concentrations of 60 μg/mL can be detected in acetonitrile. In this case a major disadvantage of the method is that the ET-30 concentrations must be precisely controlled, and a calibration curve must also be constructed for each determination.

In the examination of the polarity of aqueous micellar media, ET-30 has also been shown to be useful. Use of micellar solutions in analytical chemistry has increased in recent years and has been reviewed by Cline-Love et al. (1984). Since ET-30 is essentially insoluble in pure water, the hydrophobic interior of aqueous micelles provides an ideal site for solvation. Its insolubility in water means that partitioning between the micelles and surrounding water will not occur to a significant extent, and thus interpretation of the spectral results is simplified. Zachariasse et al. (1981) have reported the use of ET-30 as a polarity probe for micelles, microemulsions, and phospholipid bilayers. Changes in micelle conformation (e.g., sphere-to-rod transition) were easily detected by the discontinuity in measured $E_T(30)$ polarity as the concentration of sodium chloride was increased. Also, Plieninger and Baumgartel (1983) have

studied the NMR spectrum of ET-30 in various surfactant media to determine the position in which the molecule resides in the micelles. In cationic micelles the phenoxide group was found to be located in the rigid region of the electrical double layer, while in anionic micelles it is found in the diffuse layer, with the pyridinium nitrogen atom in the rigid layer.

In addition to being used as a probe of micellar environments, ET-30 also provides useful information about the structure of binary solvent mixtures. For example, Kohler et al. (1969) compared the NMR absorption spectrum for the water proton in aqueous/organic mixtures with the $E_T(30)$ polarity. Binary mixtures of water with either acetone, dioxane, or tetrahydrofuran were studied, and a linear relationship was found between the water proton absorption peak and the measured $E_T(30)$ polarity for the same mixture. It must be noted, however, that the concentration range examined was fairly small (50-95% organic component by volume), so it is possible that outside this range the relationship is not linear. Balkrishnan and Easteal (1981b) have also discussed the variation in $E_T(30)$ polarity in binary acetonitrile/water mixtures (see Chapter II).

Heats of solution at infinite dilution have been correlated with the $E_T(30)$ polarity scale by Ilic and coworkers (1984). A linear relationship was found between

a solute's $E_T(30)$ polarity and its heat of solution. Solutes that were studied included n-alkyl ketones, n-alcohols, and di-n-alkyl ethers. The heats of solution of these solutes were measured in solvents such as n-hexane, carbon tetrachloride, benzene, etc. None of the solvents were capable of hydrogen bonding with the solutes, however, and thus the results cannot be generalized to include every solute/solvent system. Also, heats of solution were measured only in pure solvents, rather than mixtures. In addition, this type of correlation would not be possible for solid compounds, since it is not possible to measure their $E_T(30)$ polarity. Of course, it might be possible to estimate the $E_T(30)$ polarity for solid compounds by using heat of solution measurements, as Fuchs and Stephenson (1983) have done for the π^* dipolarity/polarizability of solid compounds.

The $E_T(30)$ scale of solvent polarity has been applied to chromatographic systems in a number of ways. The applications discussed here include supercritical fluid chromatography and normal phase liquid chromatography (NPLC). These types of investigations can provide information about either the mobile phase (solvent polarity) or the stationary phase (surface polarity).

For example, the $E_T(30)$ polarity of a mobile phase used in supercritical-fluid chromatography (SFC) has been reported (Hyatt, 1984). Typical mobile phases used in SFC

are compressed gases such as carbon dioxide or ammonia, at a temperature greater than their critical point. Hyatt calculated the $E_T(30)$ polarity of both sub- and supercritical carbon dioxide to be 33.8 kcal/mole, by using the more lipophilic penta(tert-butyl) derivative of ET-30 (Reichardt and Harbusch-Gornert, 1983). It was necessary to use the more lipophilic compound due to the low solubility of ET-30 in supercritical CO_2. An $E_T(30)$ polarity of 33.8 kcal/mole is comparable to that of either toluene or tetrachloroethylene. However, the strength of the mobile phases used in SFC is controlled by the pressure (and resultant density). The $E_T(30)$ polarity of supercritical CO_2 was reported for only one pressure (1000 PSI) and temperature (42^oC), and thus it is likely that a different polarity would result for different pressures (densities). For example, Sigman et al. (1985) measured the π^* dipolarity/polarizability and β (hydrogen bonding basicity) for supercritical CO_2, which were found to be highly dependent on the density. Since these measurements are also based on the use of solvatochromic dyes, it is likely that the $E_T(30)$ polarity would also be greatly affected by a change in the CO_2 pressure. Thus, useful information would be provided by performing the same experiments with the more lipophilic, t-butyl derivatized betaine. Levy and Ritchey (1985) have reported on the effects of adding small amounts of additives such as

methanol or acetonitrile to the mobile phase in SFC. In theory, $E_T(30)$ polarity of these binary mixtures could also be measured.

The polarity of silica surfaces used in normal phase liquid chromatography was examined (Lindley et al., 1985). In this case, the diffuse reflectance spectrum of the betaine adsorbed onto the silica was measured. The peak corresponding to minimum reflectance was used, in conjunction with that of 4-nitroanisole, to calculate α, a measure of the acidity of the silica surface. The silica surface was found to be a strong hydrogen bond donor. The degree of the dye loading also influenced the measured values, which decreased at higher levels, apparently as a result of the formation of more than a monolayer of the test solutes on the silica surface. None of these experiments were done in the presence of a mobile phase, however, most likely because the ET-30 is quite soluble in typical mobile phases used in NPLC (such as those containing dichloromethane).

Another interesting application of $E_T(30)$ polarity measurements involves Snyder's eluent strength parameters for solvents used in normal phase liquid chromatography. Krygowski et al. (1981) compared the $E_T(30)$ polarity of various pure solvents with Snyder's eluent strength parameter (ϵ^o). In this case, it was necessary to

incorporate a second parameter, B_{KT} (Kamlet/Taft basicity), in order to predict the ε° values. Also, only pure solvents were treated, rather than the binary or ternary mixtures typically used in normal phase liquid chromatography.

To date there have been no comparisons made between empirical measurements of mobile phase polarity and chromatographic retention or selectivity. In reversed phase chromatographic experiments, it is often assumed that the strength of the mobile phase varies linearly with the percentage of organic modifier. In this dissertation, the results of empirical solvent polarity measurements of the most commonly used mobile phases are discussed, as well as the correlation between these measurements and chromatographic retention and selectivity.

CHAPTER II
SOLVATOCHROMIC SOLVENT
POLARITY MEASUREMENTS

Experimental

$E_T(30)$-Value Measurements

A sample of the ET-30 was kindly provided by Professor Christian Reichardt of Philipps-Universitat Marburg, Federal Republic of Germany. The synthesis of ET-30, which is not commercially available, is reported elsewhere (Dimroth et al., 1963b). Binary solvent mixtures were generated by a Spectra-Physics Model SP8700 ternary proportioning LC system. Degassing was achieved by sparging the solvents vigorously with helium. Both HPLC grade methanol and acetonitrile (Fisher Scientific, Fair Lawn, NJ) were used as received. Water was first purified with a Barnstead Nanopure system (Boston, MA) and then irradiated with UV light in a Photronix Model 816 H.P.L.C. reservoir (Photronix Corp., Medway, MA) for at least 24 hours. The water was then filtered through a 0.45 micrometer Nylon-66 membrane filter (Rainin Instruments, Woburn, MA) prior to use.

After collecting 3 mL of a given solvent mixture in a 1 cm path length quartz cell, approximately 0.8 mg of ET-30

was added, and a spectrum was obtained with a Hewlett-Packard Model 8450A diode array spectrophotometer. Wavelength accuracy of the instrument was checked with a Holmium Oxide interference filter. Spectra were acquired at $25\pm2^{\circ}$C. In pure methanol, the change in λ_{max} is 10 nm for a temperature change from 25 to 55°C (Dimroth et al., 1963a). Thus, a ±2 degree variation leads to an uncertainty in $E_T(30)$ values of approximately 0.1 kcal/mole. The "peak-find" function of the instrument was used to determine λ_{max}. For each solvent mixture, ten spectra were acquired at one second integration time, and the resultant λ_{max} values averaged. The pooled standard deviation for 620 λ_{max} measurements was found to be 1.16 nm. Values of $E_T(30)$ polarity were calculated from λ_{max} data by using equation 1-6.

As with many dye molecules, the possibility of dimerization of the ET-30 exists. Since $E_T(30)$ exists in a zwitterionic form, dimerization would be favored through interaction between oppositely aligned molecules. Dimerization would lead to a dependence of λ_{max} upon its concentration, as well as nonlinearity of a Beer's law plot. It has been reported that Beer's law is obeyed for concentrations in the range of 10^{-05} to 10^{-03} M (Dimroth and Reichardt, 1966); all sample concentrations were in this range. As a further check, the concentration of ET-30

in 45/35/10 MeOH/ACN/H$_2$O was varied, and λ_{max} and absorbance at λ_{max} measured. These data are shown in Table 2-1 and are plotted in Figure 2-1. No dependence of λ_{max} on ET-30 concentration was observed. Also, Beer's law was obeyed over the concentration range studied.

Table 2-1.
Effect of varying ET-30 concentration on λ_{max} and absorbance in 45/35/20 (v/v/v) MeOH/ACN/H$_2$O.

Conc. (mg/mL)	λ_{max}	Absorbance
0.13	508.5	0.2757
0.26	508.3	0.6901
0.38	508.3	1.111
0.52	509.5	1.498
0.65	508.4	1.914

Values of $E_T(30)$ have been previously reported for these same solvent mixtures (Dimroth and Reichardt, 1966; Krygowski et al., 1985); however, in these cases mixtures were prepared by adding water to the organic solvent to attain a fixed total volume. In contrast, LC pumps typically mix solvents on the basis of additive volume. For example, 100 mL of 50/50 (v/v) mixture of methanol/water (as delivered by an LC pump) is comprised of 50 mL methanol to which 50 mL water is added. Excess volumes of mixing lead to slight differences in solvent composition and resultant $E_T(30)$ polarity values, so these
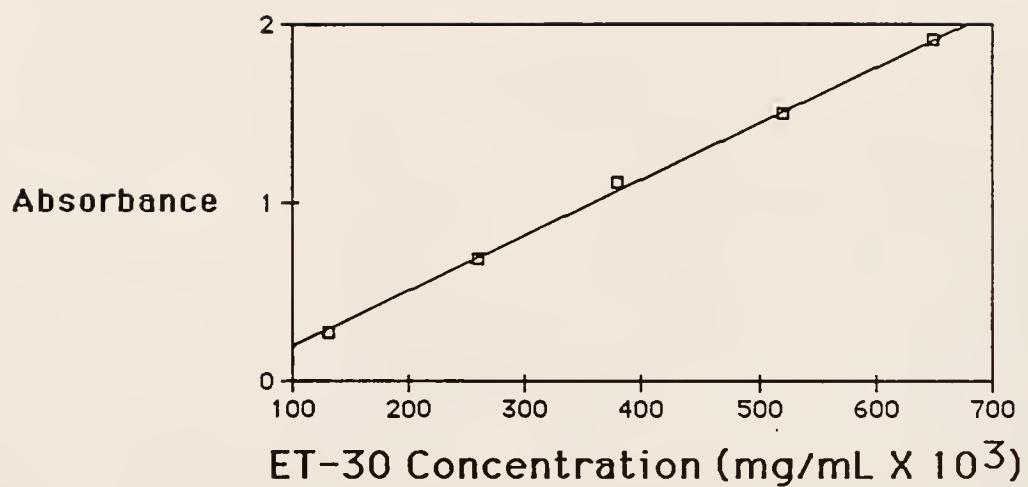
Figure 2-1. Beer's law plot for ET-30 dissolved in
45/30/10 methanol/acetonitrile/water (v/v/v).

measurements were made with solvent mixtures generated with the LC pump system itself.

π*-Value Measurements

Measurements of π* values were made with 4-nitroanisole (Aldrich Chemical Co., Milwaukee, WI) and using the following equation from Kamlet et al. (1977):

$$\pi^* = (\nu_{max} - \nu_0)/2.343 \qquad (2\text{-}1)$$

where ν_{max} is the observed maximum in wavenumbers (X 10^{-03} cm^{-1}), and ν_0 is the value for the solute in cyclohexane ($\pi^* = 0$ in cyclohexane, by definition). This reference (Kamlet et al., 1977) lists a number of solutes (for example, 4-ethylnitrobenzene) that can be used to measure the π* dipolarity/polarizability; 4-nitroanisole was chosen because of its low sensitivity to hydrogen bond donor/acceptor effects. In this case the 4-nitroanisole was added to the water at a concentration of 5 µg/mL, and the resulting solvent mixture + solute was passed through a 0.25 mL Hellma flow cell thermostatted at 40±0.1°C with a Haake Model D1 water bath (Haake, Saddle Brook, NJ). Flow was stopped while acquiring spectra to equilibrate the temperature of the mixture and reduce the effect of refractive index variability in the sample.

Because of the very small wavelength shift observed with this substance in going from pure organic to pure

water (λ_{max} of 9 nm between water and acetonitrile), the following algorithm was used to evaluate λ_{max}. Spectra were acquired, and the absorbance recorded at each wavelength (1 nm readout resolution). Next the absorbance data were fit with a 3rd degree polynomial using the program "Curve Fitter" (see Appendix C; this algorithm was suggested by Savitizky and Golay, 1964). The first derivative (dy/dx) of the resultant polynomial was then used to evaluate λ_{max} (by setting this equal to zero and solving for λ_{max} with the quadratic formula). Repeated calculations with either the entire data set (30 points; 30 nm wide) or only five points ($\lambda_{max}{}^{-10}$, $\lambda_{max}{}^{-5}$, λ_{max}, $\lambda_{max}{}^{+5}$, $\lambda_{max}{}^{+10}$) showed that only five were needed to define the spectral peak accurately. By interpolating the spectral peak position in this manner, the precision of λ_{max} measurement was greatly improved. As an illustration of the utility of this algorithm, in Table 2-2 and Figures 2-2 and 2-3, the effect of temperature on the peak position of 4-nitroanisole in 33.3/33.3/33.4 (v/v/v) MeOH/ACN/H_2O is shown. Data of λ_{max} provided directly by the instrument or that from interpolation (of the same spectral data set) are plotted in Figures 2-2 and 2-3, respectively. The data clearly indicate that thermochromism of 4-nitroanisole is not observable without the use of this algorithm.
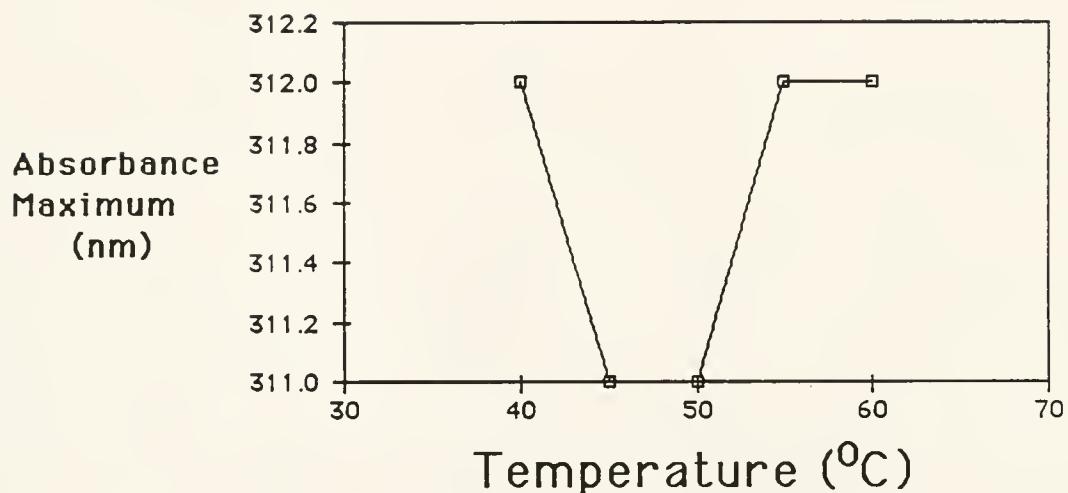
Figure 2-2.   Thermochromism of 4-nitroanisole in 33.3/33.3/33.4 methanol/water/acetonitrile (v/v/v).   Wavelengths obtained directly from the diode array spectrophotometer.
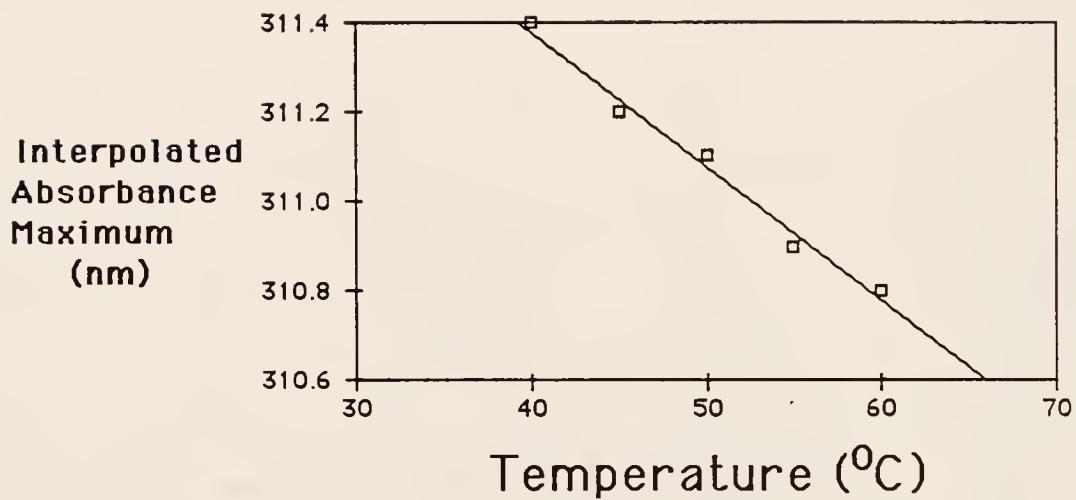
Figure 2-3.   Thermochromism of 4-nitroanisole in 33.3/33.3/33.4 methanol/water/acetonitrile (v/v/v). Wavelengths obtained by interpolation of absorbance data from the diode array spectrophotometer.

42

Table 2-2.
Thermochromism of 4-nitroanisole in
33.3/33.3/33.4 (v/v/v) MeOH/ACN/H$_2$O.

Temperature ($^{\circ}$C)	λ_{max} directly	λ_{max} (interpolated)
40.0	312	311.4
45.0	311	311.2
50.0	311	311.1
55.0	312	310.9
60.0	312	310.8

Spectra of 4-nitroethylbenzene and 4-nitrophenol were also acquired in the same manner in methanol/water and acetonitrile/water mixtures, in connection with the measurement of solvent α and β values (not utilized in the present discussion; results tabulated in Appendix D).

Results

π*-Values

While the primary purpose of this research was to investigate the E$_T$(30) polarity scale in regard to chromatographic retention, measurements were also done for the π* scale of solvent dipolarity/polarizability in binary hydro-organic mobile phases.

In Figure 2-4, a representative spectrum for 4-nitro-anisole in methanol is shown. One advantage of the use of this scale is that the spectral peak of interest (due to the nitro group) is widely separated from that of the

43
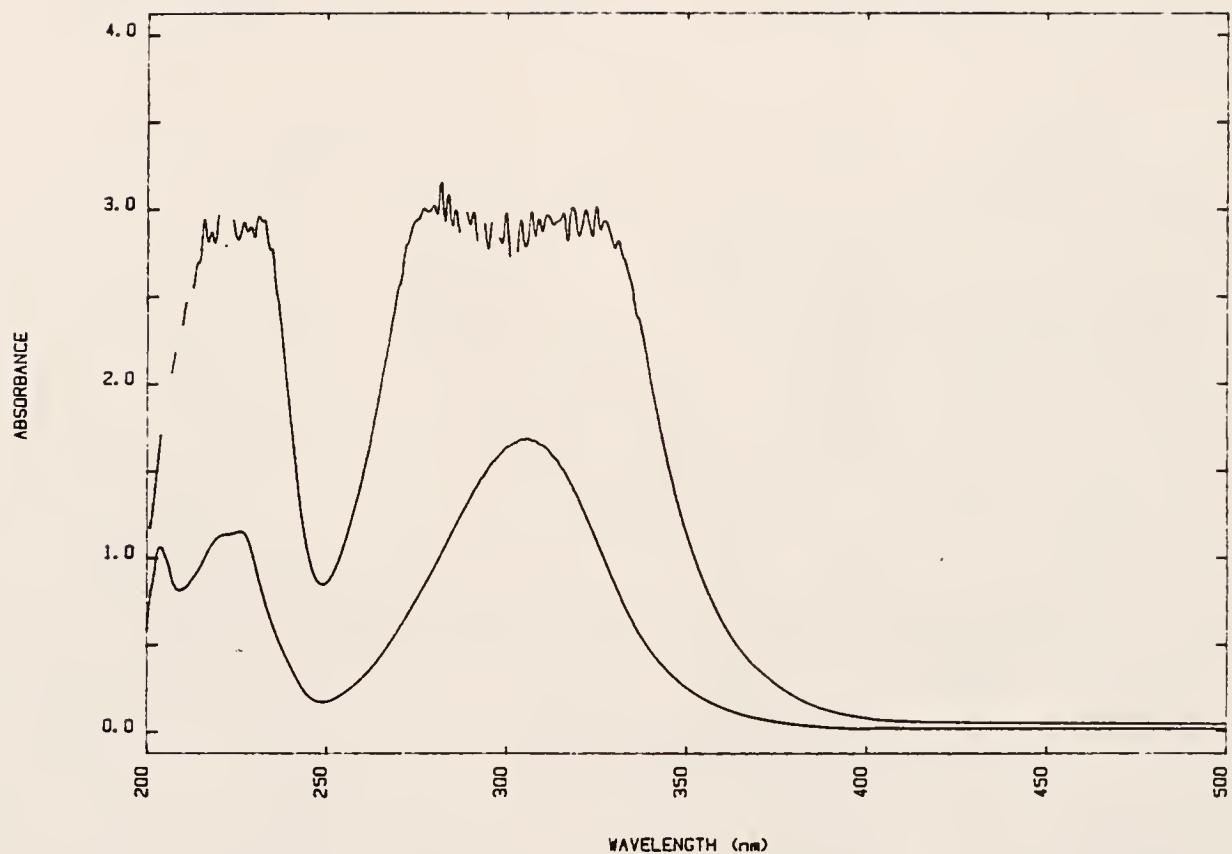
Figure 2-4. Representative UV/VIS absorbance spectrum of 4-nitroanisole in methanol. Concentrations for the two curves are top, 0.1 mg/mL; bottom, 0.02 mg/mL.

aromatic π-electron system.   As discussed in Chapter I,
overlap of peaks can be a problem, as best exemplified with
Z-values (Kosower, 1958), in which the charge transfer peak
merges with that of the pyridine ring in highly aqueous
mixtures.

The   results   of   π*   dipolarity/polarizability
measurements for binary methanol/water mixtures appear in
Figures 2-5 and 2-6.   In terms of percentage methanol
(Figure 2-5), the π* values are seen to decrease steadily,
in a highly nonlinear fashion.   However, when the data are
plotted versus mole fraction of methanol (Figure 2-6), a
nearly straight line results (r^2 = 0.9959, s = 0.0119).

In Figures 2-7 and 2-8, the corresponding measurements
for the acetonitrile/water solvent mixtures are depicted.
Here the variation is much more complex; this is especially
true when compared to percentage of acetonitrile (Figure
2-7), where there are at least two points of inflection at
approximately the 30 and 70% concentrations.   In contrast
to methanol/water mixtures, the variation with respect to
mole fraction of acetonitrile (Figure 2-8) is seen to be
highly nonlinear.

The π* scale of solvent dipolarity/polarizability is
distinctly different from the $E_T(30)$ scale in that it is
specifically   intended   to   exclude   hydrogen   bond
donor/acceptor effects.   As such these results then show
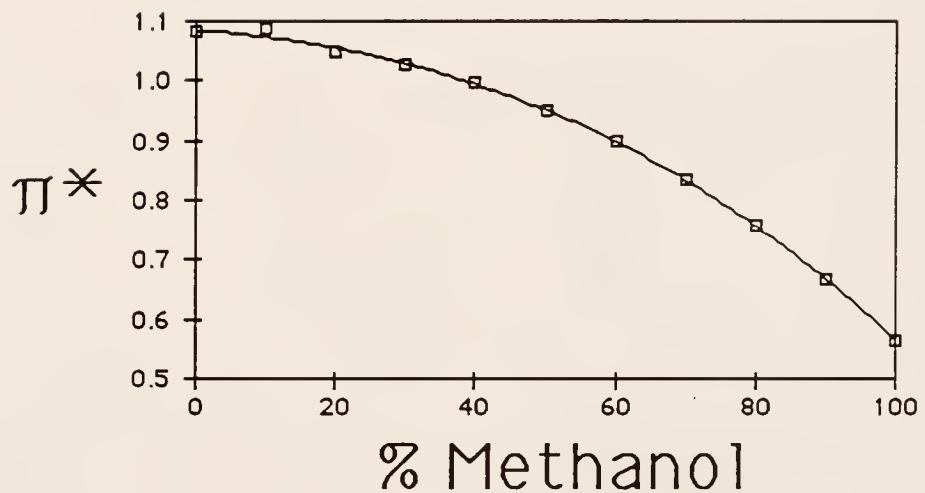
Figure 2-5. Measurements of π^* dipolarity/polarizability for methanol/water mixtures with respect to percent methanol.

46
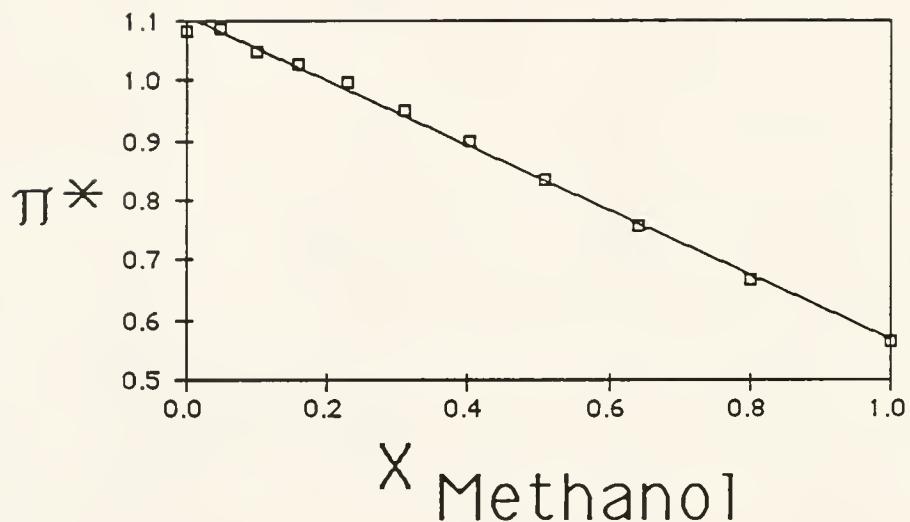
Figure 2-6. Measurements of π* dipolarity/polarizability for methanol/water mixtures with respect to mole fraction of methanol.
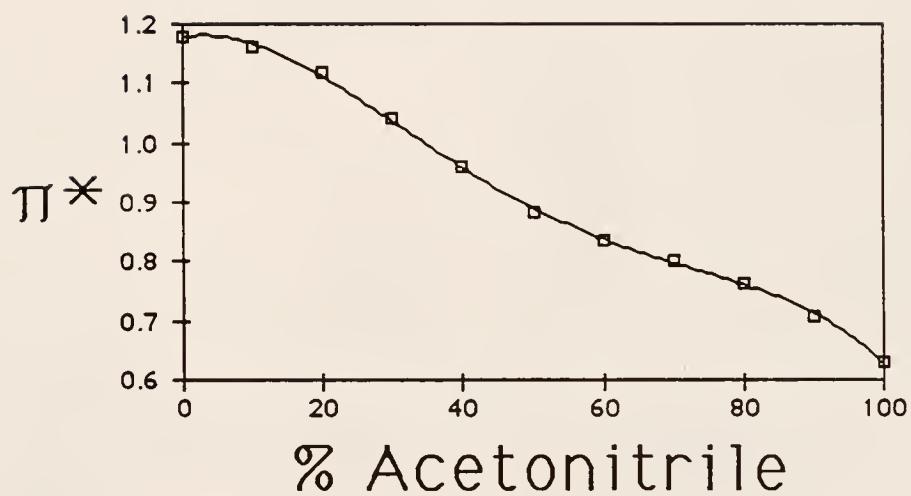
Figure 2-7. Measurements of π* dipolarity/polarizability for acetonitrile/water mixtures with respect to percent acetonitrile.

48
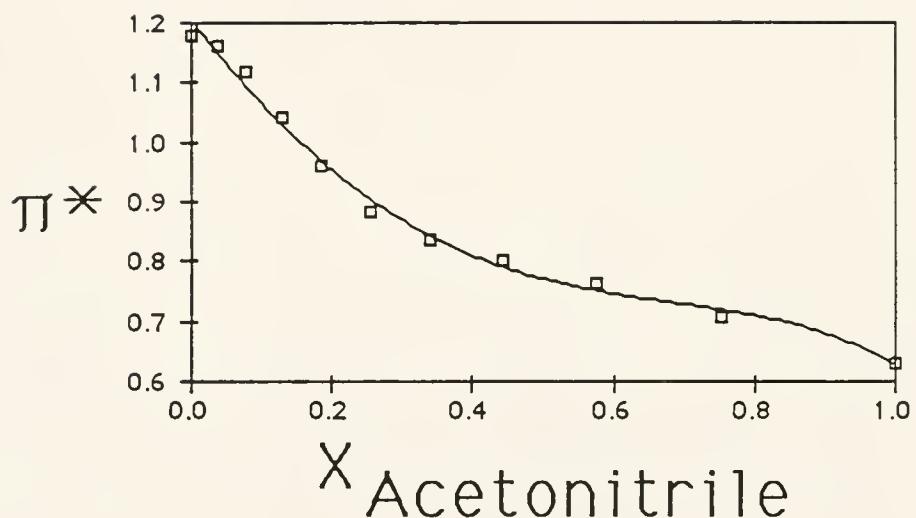
Figure 2-8.   Measurements of π* dipolarity/polarizability
for acetonitrile/water mixtures with respect
to mole fraction of acetonitrile.

the variation in polarity due only to dipole/dipole, dipole/induced dipole, and dispersion interactions. Thus it is not surprising that at 100% organic concentration, methanol is actually less polar than acetonitrile (π^* values of 0.57 and 0.67, respectively). This is a direct reflection of the fact that the nitrile bond of acetonitrile is much more dipolar in nature than either the C-O or O-H bonds of methanol. Kamlet et al. (1983) have reported π^* values of 0.60 and 0.75 for methanol and acetonitrile, respectively. These compare with the values reported here of 0.57 and 0.67 for the corresponding solvents. This discrepancy between the values reported by Kamlet et al. and shown here is not significant, however. In the present work, π^* values were calculated from measurements obtained with one solute (4-nitroanisole), while those reported by Kamlet et al. (1983) are actually the values that lead to the most consistent result from several test solutes. In fact, in the original paper describing the π^* scale, Kamlet et al. (1977) reported values of 0.58 and 0.71 for methanol and acetonitrile, respectively.

Based solely on the π^* scale, one would conclude that methanol should be a stronger (less polar) organic modifier for RPLC. However, this conclusion does not agree with the known properties of the two organic modifiers, since acetonitrile behaves as a more nonpolar, hence stronger,

solvent in RPLC. Also, one would expect (based on π^* values) that methanol would solvate the stationary phase alkyl chains to a greater extent, which, again, is simply not consistent with the known properties of the two modifiers (as discussed in Chapters I and V).

$E_T(30)$-Values

A representative spectrum for ET-30 dissolved in pure methanol is shown in Figure 2-9. The very large absorption at wavelengths less than 400 nm is due to the aromatic π-electron system. In pure water, λ_{max} decreases to 453 nm (Dimroth et al., 1963a; a 10 cm path length cell was used). It was not possible to obtain spectra of $E_T(30)$ in pure water (due to its extremely low solubility; $<10^{-06}$ M), so this value has been used in the following figures.

In Figures 2-10 and 2-11, the $E_T(30)$-values are plotted with respect to percent and mole fraction of methanol, respectively. In Figures 2-12 and 2-13, the corresponding results for acetonitrile/water mixtures are shown.

With both organic modifiers, the $E_T(30)$ polarity is clearly a nonlinear function of composition; this is not surprising since none of the solvents form thermo-dynamically ideal solutions. For a thermodynamically ideal binary solvent mixture, any bulk physical property, such a dielectric constant or viscosity, is expected to be a
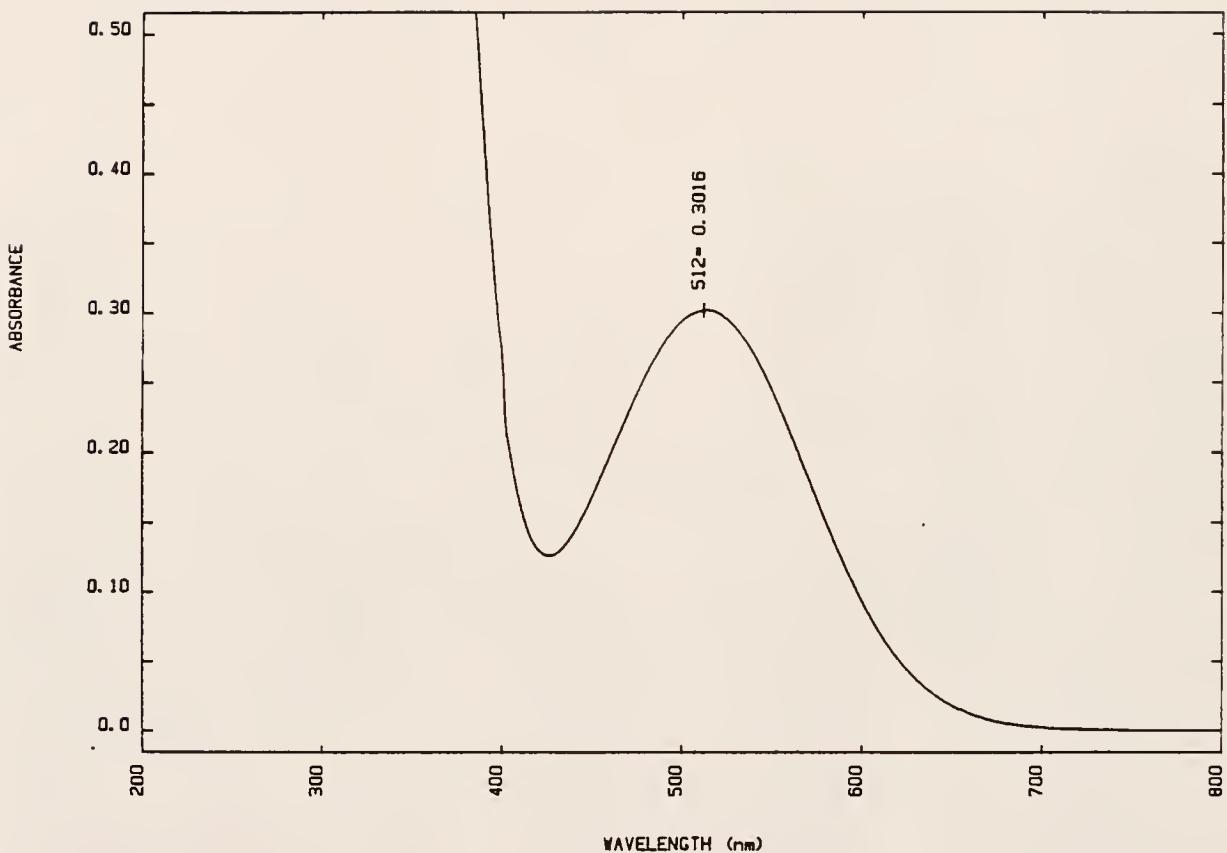
Figure 2-9. Representative UV/VIS absorption spectrum of the ET-30 dye dissolved in methanol.
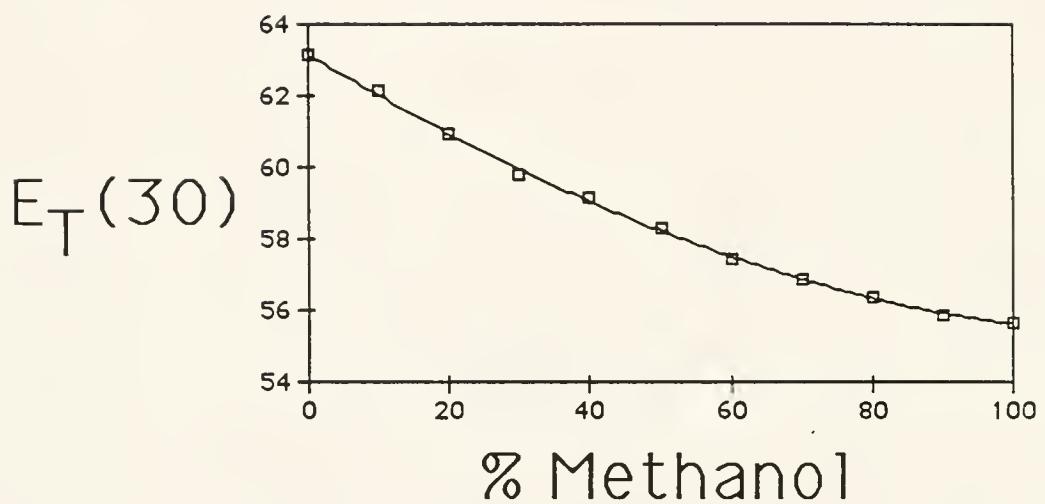
Figure 2-10.   Measurements of $E_T(30)$ polarity for methanol/
water   mixtures   with   respect   to   percent
methanol.
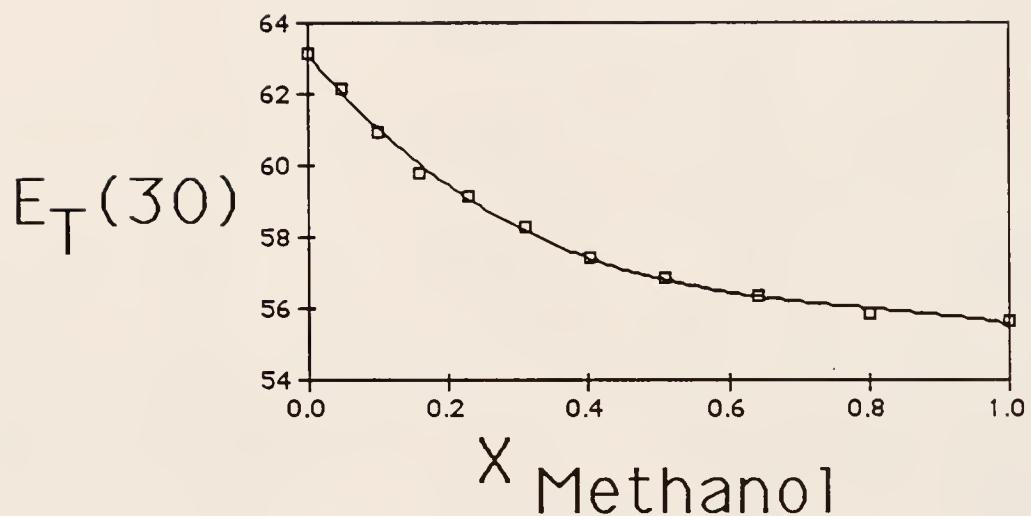
Figure 2-11. Measurements of $E_T(30)$ polarity for methanol/
water mixtures with respect to mole fraction
of methanol.
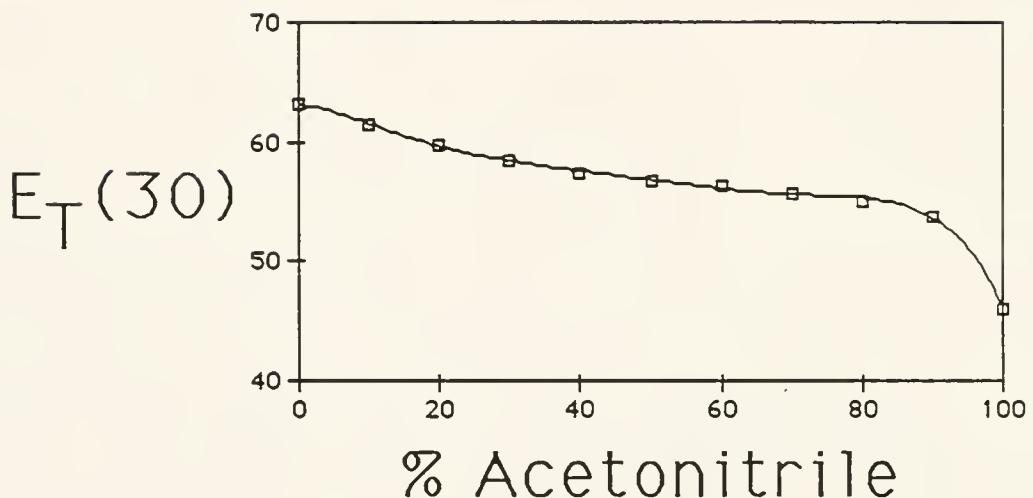
Figure 2-12.   Measurements of $E_T(30)$ polarity for aceto-
nitrile/water mixtures with respect to
percent acetonitrile.
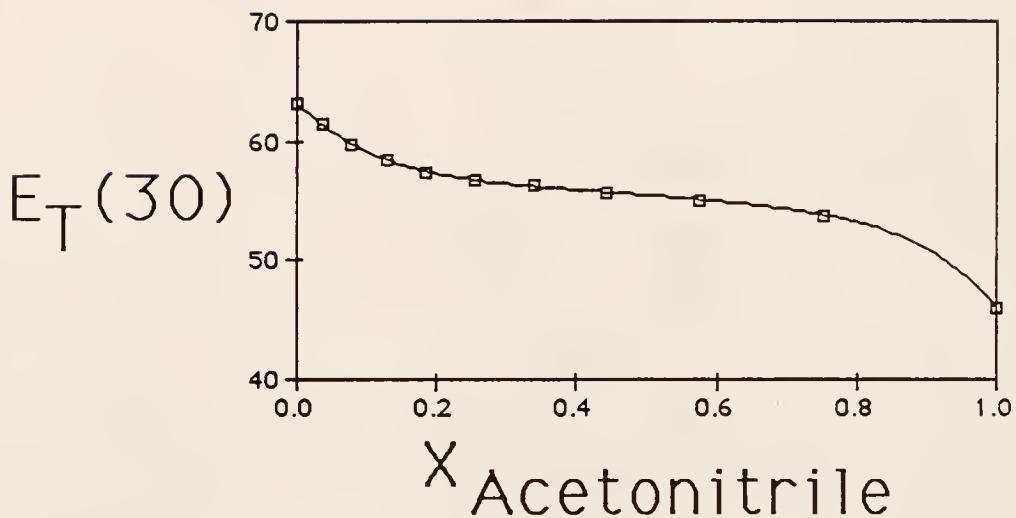
Figure 2-13. Measurements of $E_T(30)$ polarity for aceto-
nitrile/water mixtures with respect to mole
fraction of acetonitrile.

linear function of the mole fraction of either component. This is also the case for empirical solvent polarity measurements. For example, this was reported to be true for the $E_T(30)$ polarity of binary mixtures of 1,2-dibromoethane and 1,2-dibromopropane, whose mixtures obey Raoult's law, demonstrating ideal solution behavior (Balakrishnan and Easteal, 1981a).

That methanol/water and acetonitrile/water mixtures are not ideal solutions is also evidenced by the nonlinear variation in viscosity and dielectric constant (Horvath and Melander, 1977). Thus, it is not surprising that the measured $E_T(30)$ polarity varies in a highly nonlinear manner versus either percent or mole fraction of organic component. The nonlinearity of these diverse properties also illustrates the danger of assuming strictly additive solvent properties, as is done in the derivation of both liquid chromatographic retention models and gradient elution schemes. It should also be pointed out here that in gas chromatography, blending of stationary phase materials can be done with this assumption in mind. This is a reflection of the fact that these phases are almost always nonpolar or weakly polar, nonhydrogen bonding materials, and thus mixtures are nearly ideal in a thermodynamic sense (Chien et al., 1980). Also, the mobile phases used in gas chromatography are nearly inert gases

(hydrogen, helium, or nitrogen) and do not solvate the stationary phase.

It is apparent that the variation in polarity of the two systems is quite different. The different character of these curves is a reflection of the differing hydrogen bonding abilities of the two organic solvents. In the case of acetonitrile, it is obvious that for concentrations greater than 80% (by volume), the measured polarity decreases rapidly.

Balakrishnan and Easteal (1981b) have discussed the variation in $E_T(30)$ polarity in acetonitrile/water mixtures and have found it to be consistent with the Naberukhin-Rogov model (1971) for binary mixtures of water with a nonelectrolyte. The Naberukhin-Rogov model describes the structure in terms of two microphases (α and β) at concentrations of greater than 0.15 (mole fraction) of acetonitrile. The α phase consists primarily of highly structured water, while microphase β contains mostly acetonitrile. At concentrations of greater than 0.6, Balakrishnan and Easteal (1981b) postulated that the β microphase predominates, and the water exists as single molecules coordinated to these "globules" of acetonitrile. Further evidence of the existence of microphases is the phase separation that occurs in this system, at a critical temperature and concentration of 272 K and 38 mole % acetonitrile, respectively.

Unlike methanol, acetonitrile is a very weak hydrogen bond donor solvent and thus as the concentration is increased, the remaining water becomes specifically associated with the ET-30 due to the presence of the negatively charged phenoxide group. As the water is completely removed, and the ET-30 is no longer stabilized through this hydrogen-bonded network, the apparent polarity plunges 46.0 kcal/mole. This large change in $E_T(30)$ polarity is not mirrored by the changes seen in log k' retention measurements. Retention data for concentrations greater than 80% have not been included in the present data analysis. There were 61 cases among these data sets where the 90% acetonitrile point was not included; these were all from one reference (Hanai and Hubert, 1983). It must also be pointed out, however, that at these concentrations the retention time will be very short for most solutes, so that the resultant k' (approaching zero) and log k' values (approaching minus infinity) will have the highest relative uncertainty of the entire retention data set. In fact, the average log k' for the 61 (90% acetonitrile) points not included was 0.049 (s = 0.089), corresponding to an average k' of 1.12.

Relationship Between Snyder's P' Polarity Values and the $E_T(30)$ Scale

Snyder (1974, 1978) has devised the P' scale of solvent polarity for use in characterizing solvents used in liquid chromatography. These values are based on gas/liquid partition coefficients for various solutes and solvents reported in the literature (Rohrschneider, 1973). For each solvent, the logarithm of the corrected partition coefficients (K"; corrected to account for differences in molecular volume and concentration units) for ethanol, dioxane, and nitromethane are summed together to calculate a P' polarity as shown below:

$$P' = \log K''(1,4\text{-dioxane}) + \log K''(\text{ethanol})$$

$$+ \log K''(\text{nitromethane}) \qquad (2\text{-}2)$$

In this manner, the solvent's ability to undergo three types of interactions (proton donor/acceptor, polar) with solutes is measured, and P' values then represent the total of these potential interactions. Snyder also reported the fractional contribution of each of the three test solutes (X_e, X_d, X_n parameters) to the overall P' value. Using these partial contribution values, Snyder classified all solvents into eight possible categories. This classification is often referred to as Snyder's solvent

selectivity triangle, in that three characteristics (proton donor, proton acceptor, and polar) are assigned to each of the three vertices of a triangle). Each solvent can then be placed into a unique position within this triangle on the basis of its X_e, X_d, and X_n values.

Since Snyder's classification scheme is intended to be useful for the measurement of solvent selectivity in liquid chromatography, it is worthwhile to examine briefly the relationship (if any) between the P' and $E_T(30)$ scales of solvent polarity. The relationship between Snyder's eluent strength parameters for NPLC and $E_T(30)$ polarity has already been discussed in Chapter I.

The easiest comparison that can be made is between the P' (summed polarity) values reported by Snyder (1978) and $E_T(30)$ polarity values for pure solvents reported by Reichardt and Harbusch-Gornert (1983). There were 48 cases in which this comparison could be made; the resultant comparison plot is shown in Figure 2-14. While there is a statistically significant correlation between the two sets (r = 0.7986), there is also a great deal of scatter around the line (s = 1.1146), so $E_T(30)$ values cannot be used to accurately predict P' values or vice versa. The line drawn through the data (using linear regression) in Figure 2-14 has a slope of 0.1861±0.04 and y-intercept of -3.54±1.84.

That there is such a poor correlation is not surprising, since the P' values represent the summation of
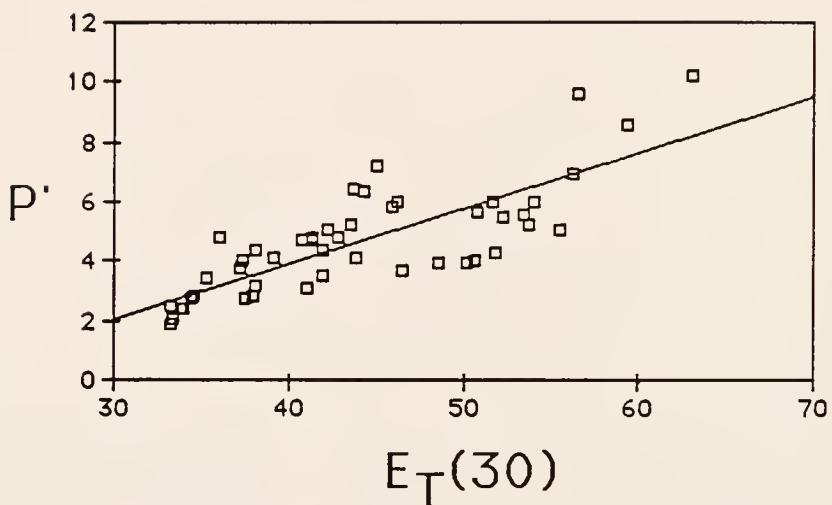
Figure 2-14.   Comparison between Snyder's P' and Dimroth-
Reichardt's $E_T(30)$ polarity values for pure
solvents.

the three interactions in proportions that will not necessarily be similar to the responsiveness of the ET-30 probe. Also, it should be noted that according to the P' scale, methanol is a stronger organic modifier for RPLC (P' = 5.1) than acetonitrile (P' = 5.8), which is not in agreement with the known chromatographic properties of these two solvents.

Perhaps a better way to compare these scales is to compare $E_T(30)$ polarity values with the partial contribution values (X_e, X_d, and X_n) by using multiple linear regression. This should allow the various contributions to be more properly weighted. However, it must be remembered that these partial values represent the fraction of the total P' value for each solvent and will always add up to one. Thus, the true magnitude of each of the three interactions is masked, and to make a valid comparison, one must first multiply each partial contribution value by the total P' value for each solvent. Using multiple linear regression, an attempt was made to correlate each $E_T(30)$ value with the three corrected partial contributions for the same solvent. For the 48 cases, the multiple correlation coefficient was found to be 0.8912, with a standard deviation of 3.685. The equation relating the $E_T(30)$ to the three interactions was

$$E_T(30) = 29.9 \pm 3.8 + 7.83 \pm 1.8\ X'_e + 2.8 \pm 2.6\ X'_d$$

$$- 1.79 \pm 2.9\ X'_n \qquad\qquad (2\text{-}3)$$

The regression coefficients indicate that the $E_T(30)$ scale is significantly related to only the terms derived from the partition coefficients for ethanol and dioxane. These results are shown graphically in Figure 2-15, where the $E_T(30)$ values predicted by equation 2-3 are plotted with respect to actual reported $E_T(30)$ polarity values (Reichardt and Harbusch-Gornert, 1983). It is interesting to note that this multiple linear regression leads to a poorer standard deviation (s = 3.86) than obtained by plotting the original P' versus $E_T(30)$ values (s = 1.11).
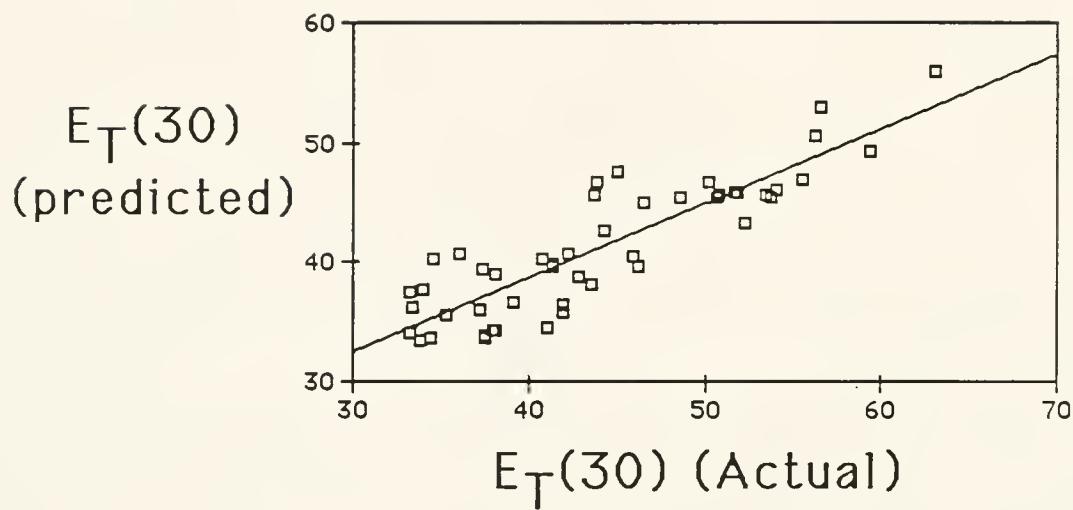
Figure 2-15. Comparison between $E_T(30)$ polarity values predicted by equation 2-3 and actual $E_T(30)$ polarity values reported by Reichardt and Harbusch-Gornert (1983).

CHAPTER III
CORRELATIONS BETWEEN CHROMATOGRAPHIC
RETENTION AND MOBILE PHASE POLARITY

Experimental

Retention measurements (other than those reported in the literature) were obtained with a Spectra-Physics SP8700 ternary proportioning LC system (Spectra-Physics, San Jose, CA). Columns were an Altex Ultrasphere ODS (5 micron particle size; Altex Scientific, San Ramon, CA) and a Hamilton PRP-1 (10 micron; Hamilton Company, Reno, NV). Both columns were of size 15 cm X 4.6 mm I.D. Test solutes were obtained from Aldrich Chemical Co. (Milwaukee, WI) and the Eastman Kodak Co. (Rochester, NY). Sample introduction was achieved with either an Altex injector equipped with a 5 microliter sample loop (Altex Scientific, San Ramon, CA) or a Rheodyne Model 7125 injector equipped with a 20 microliter sample loop (Rheodyne, Inc., Cotati, CA). Flow rates were either 1.0 or 2.0 mL/min. The column was thermostatted at $40\pm0.1^{\circ}C$ with a Haake Model D1 water bath (Haake, Saddle Brook, NJ). Solvents were obtained as described previously (Experimental, Chapter II). A fixed wavelength, 254 nm, Beckman Model 153 UV detector (Altex Scientific, San Ramon, CA) was used.

The retention times for an unretained species (t_o) were evaluated with injections of the pure organic solvent (either methanol or acetonitrile). For the Hamilton PRP-1 column, this proved to be difficult at low organic modifier concentrations due to actual retention of the acetonitrile or methanol. Other supposedly unretained solutes (such as urea and uracil) exhibited similar behavior. Therefore, the t_o obtained from injections of pure organic modifier at 60% organic modifier concentration was used, since at this concentration the retention time reached a minimum in each of the two solvent systems.

Simple linear regression calculations were done with the program "Curve Fitter" (Interactive Microware, Inc., State College, PA) run on an Apple II Plus 48K microcomputer (Apple Computer, Inc., Cupertino, CA). The program was modified to allow calculation of 95% confidence intervals for slope and y-intercept values. This program was also used to interpolate $E_T(30)$ values for solvent compositions that had not been measured (e.g., 45% methanol/water).

When curve fitting the data to either a linear or 2nd degree polynomial, the resultant standard deviations (s-values) were used to calculated an F-ratio as

$$F = s(\text{linear})/s(\text{2nd degree polynomial}) \qquad (3\text{-}1)$$

The significance level ($\alpha\%$ values reported in Table 3-1) of a given F-ratio was then determined by using the program "F Distribution" (public domain software provided by Computer Learning Center, Tacoma, WA). In this way, much more accurate estimates of the significance level were obtained than those from published F-distribution statistical tables.

Multiple linear regression calculations were done by using the program "Statworks" (Datametrics, Inc., and Heyden and Son Limited, Philadelphia, PA), run on a Macintosh 512K computer (Apple Computer, Inc., Cupertino, CA).

Results

In RPLC, retention of solutes decreases as the concentration of organic modifier is increased. That is, as the overall polarity of the mobile phase is decreased, solutes will spend less time in the stationary phase. Of course, there are many ways to express this decrease in polarity; the simplest measure of this is the proportion of the organic modifier. Traditionally, chromatographers have measured capacity factors at various organic modifier concentrations and then plotted the logarithm (base 10) of the capacity factor as a function of this concentration. In the present discussion, the abbreviation "log" shall denote the base ten logarithm. Plotting the logarithm of

capacity factor is quite logical, owing to its dependence on the free energy of transfer (ΔG) of the solute between the mobile and stationary phases. This relationship is expressed by the following equation:

$$\log k' = (-2.303\ \Delta G/RT) + \log(\phi) \qquad (3\text{-}2)$$

where ϕ is the phase ratio for the particular column. Thus, plotting log k' versus percent organic modifier gives a sense of the change in the energetics of chromatographic retention as the composition of the mobile phase is changed. Whether or not this type of plot is linear in nature has been the subject of much debate. In terms of concentration, the only reason that percent organic modifier is usually used is that all chromatographic instrumentation has been built to deliver mixtures by volume percentage. While plots of log k' versus percent organic modifier often appear to be linear, they will always exhibit some curvature if a wide enough concentration range is investigated and are best fit by a quadratic equation (Schoenmakers et al., 1983). To illustrate this point, in Figure 3-1, retention data for 4-nitrophenol have been plotted with respect to percent organic modifier. If the data are fitted with a straight line, a squared correlation coefficient of 0.9803 is found, while a quadratic curve-fitting leads to an increase to

0.9983. Clearly, the variation in the log k' values is best accounted for by an equation containing a quadratic term.

From a physical standpoint, it would be much more logical to plot the retention data with respect to the mole fraction of organic modifier. That is, solution properties (of which reversed phase chromatographic retention can be considered to be a result of) are best expressed by observing the property as a function of mole fraction. In such cases, deviations from linearity are then (by definition) deviations from nonideal solution behavior.

The extent to which plotting log k' values versus either volume percent or mole fraction or organic modifier can affect the curve shape is illustrated in Figures 3-2 and 3-3. For both methanol and acetonitrile, the mole fraction has been plotted with respect to percent of organic modifier. In both cases, the actual mole percent is significantly lower than the percent by volume at all concentrations (except, of course, at the 0 and 100% points). Using the same log k' values shown in Figure 3-1, the data have been re-plotted with respect to the mole fraction of acetonitrile in Figure 3-4. Here the curvature has been accentuated, and a straight line fit of the data yields a r^2 of 0.9332. Since the variation in mobile phase strength is not necessarily directly related to the percent (by volume) or the mole fraction, a more logical approach

would be to compare retention with experimentally derived measures of mobile phase polarity, such as the π^* and $E_T(30)$ values. In Figure 3-5, log k' values for 4-nitrophenol (same values as used in Figures 3-1 and 3-4) are plotted with respect to the π^* values for the same composition (π^*-values discussed in Chapter II). In this case, there is a point of inflection, and the data are best fit by a 3rd degree polynomial. As discussed in Chapter II, one would not expect the π^* scale to correlate well with chromatographic retention, owing to its insensitivity (by design) to hydrogen bonding effects in solution. This is clearly reflected by the data shown in Figure 3-5. A straight line fit of the data results in a squared correlation coefficient of 0.9667.

The $E_T(30)$ values discussed in Chapter II can also be compared with chromatographic retention. The $E_T(30)$ scale has been shown to be sensitive to both hydrogen bonding and dipolarity effects (as discussed in Chapter I) and thus may serve as a better indicator of the strength of the mobile phases used in RPLC. In Figure 3-6, retention data used in previous figures have been plotted with respect to the measured $E_T(30)$ polarity for the same mobile phase composition. In this case, the linearity is much greater, yielding a square correlation coefficient of 0.9950 when fitted to a straight line model. This is in great contrast
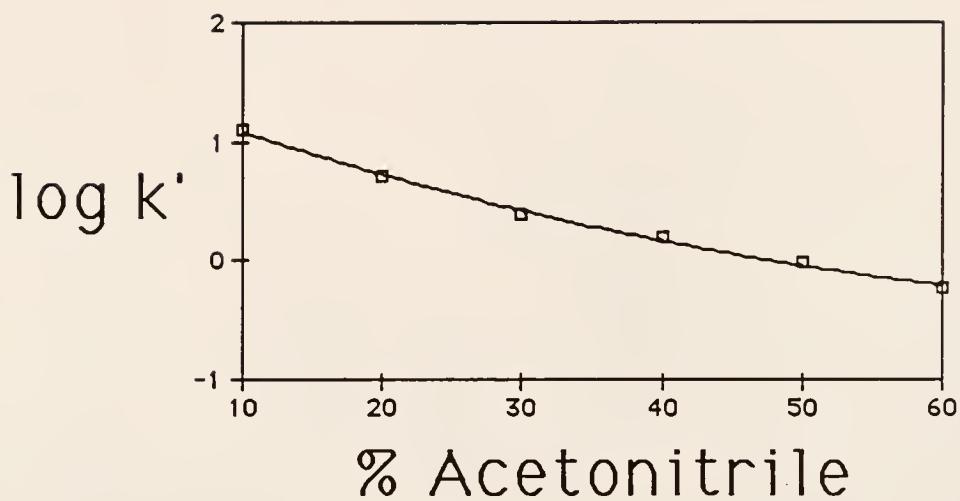
Figure 3-1.   Retention data for 4-nitrophenol plotted with
              respect to percent acetonitrile.   Ultrasphere
              ODS (C-18) column; flow rate 1.0 mL/min.
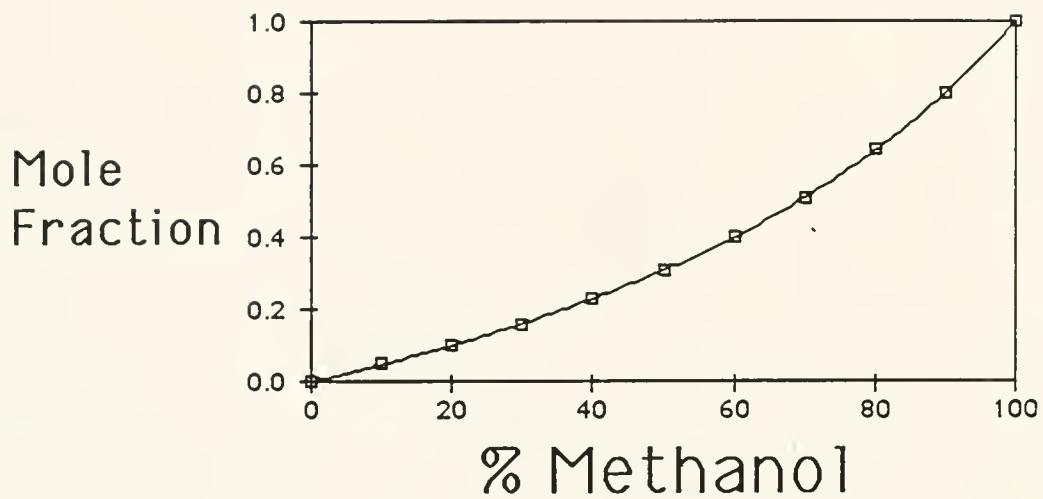
Figure 3-2. Variation in mole fraction of methanol as a function of volume percent.
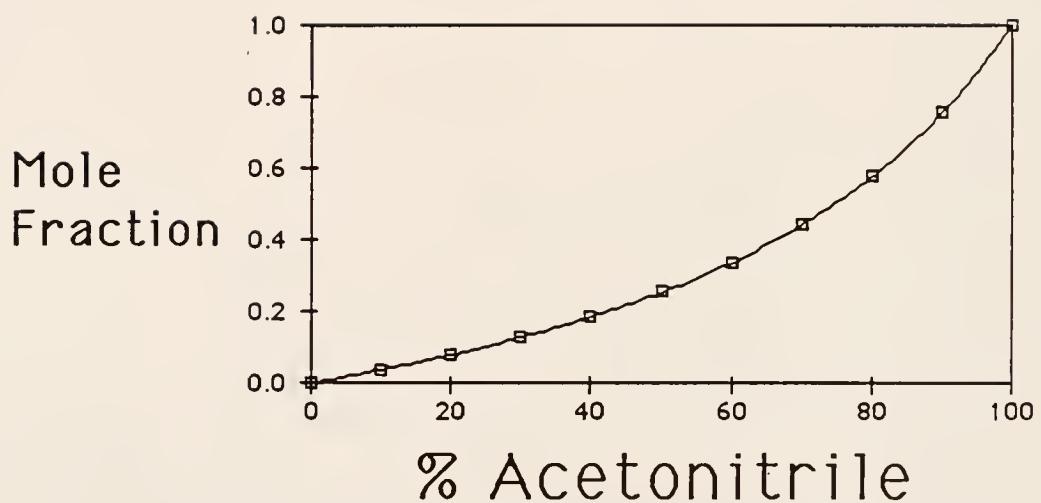
Figure 3-3.   Variation in mole fraction of acetonitrile as
             a function of volume percent.
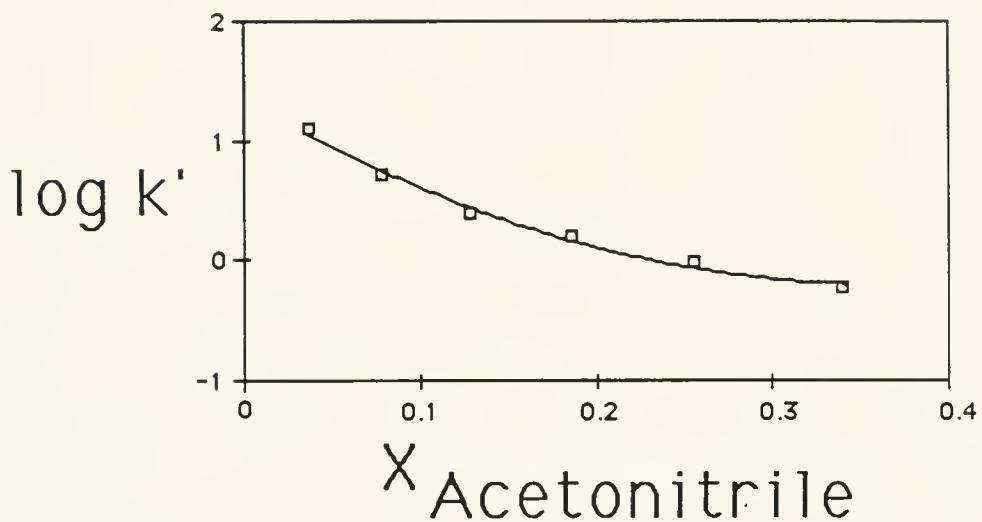
Figure 3-4.   Retention data for 4-nitrophenol plotted with respect to mole fraction of acetonitrile. Ultrasphere ODS (C-18) column; flow rate 1.0 mL/min.
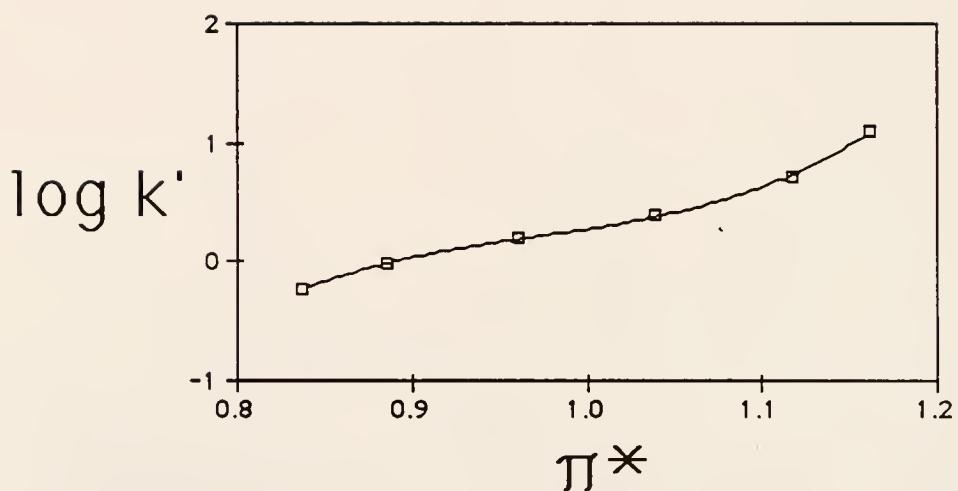
Figure 3-5.   Retention data for 4-nitrophenol plotted with
              respect to π* dipolarity/polarizability for
              the same solvent mixtures.   Ultrasphere ODS
              (C-18) column; flow rate 1.0 mL/min.
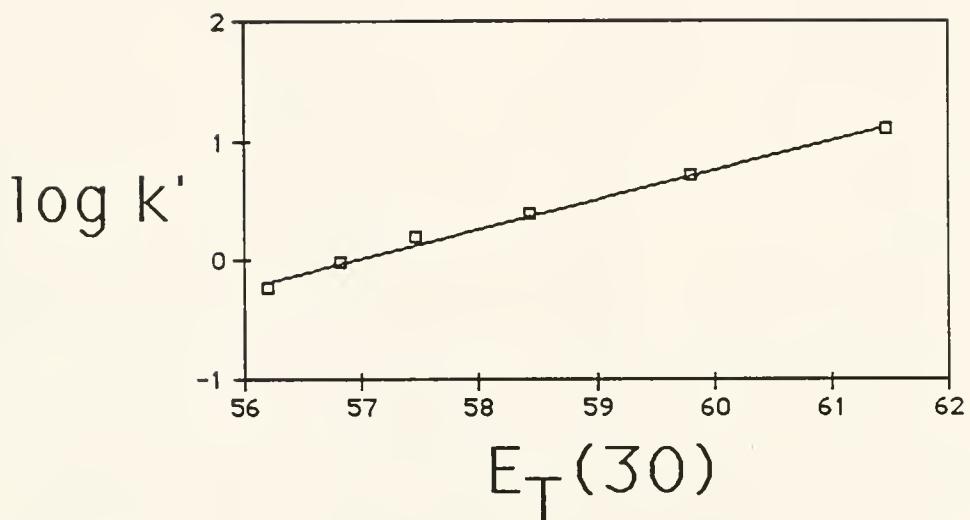
Figure 3-6. Retention data for 4-nitrophenol plotted with respect to the $E_T(30)$ polarity for the same solvent mixtures.   Ultrasphere ODS (C-18) column; flow rate 1.0 mL/min.

to the other three values for Figures 3-1, 3-4, and 3-5, of
0.9803, 0.9332, and 0.9667. The best fit is obtained when
the log k' values are plotted with respect to the measured
$E_T(30)$ polarity for the same mobile phase mixture.

Of course, the previous figures pertain to only one
individual set of retention data generated for this
research; in order to make any generalizations about the
correlations between the various variables, it is necessary
to examine a large body of chromatographic data. A total
of 332 sets of chromatographic retention data (log k'
versus percent organic modifier) have been examined.
Retention data reported in the literature, as well as data
generated exclusively for this study, have been included in
these correlations. Hereafter the discussion will be
confined to two types of correlations: those between log
k' and either percent organic modifier or $E_T(30)$ polarity.

Linear regression was carried out for all retention
data sets with the log k' data compared to both percent
organic modifier and the $E_T(30)$ polarity. The results of
these correlations are compiled in Table 3-1. The data
have been sorted in a hierarchical manner, using the
following sequence: organic modifier, column, and
solute. Squared correlation coefficients (r^2) for both log
k' versus organic modifier and $E_T(30)$ polarity are
reported, as well as the regression coefficients for the

Table 3-1.
Linear regression results for correlations between log k' and either percent organic modifier or $E_T(30)$ polarity.

Date	Solute	[a]Column	Solvent/% Range	r^2 vs. %	r^2 vs. $E_T(30)$	Slope($\times10^2$)	$-(y\text{-int})$	σ	n	[b]α	Reference
1	2-Nitroaniline	A	ACN/10-60	0.9869	0.9909	23.4±3.1	13.2±1.8	0.0498	6		This Work
2	4-Nitroaniline	A	ACN/10-60	0.9865	0.9903	20.8±2.8	11.9±1.7	0.0456	6		This Work
3	4-Nitrophenol	A	ACN/10-60	0.9803	0.9948	24.5±2.4	13.9±1.4	0.0391	6		This Work
4	4-Nitroanisole	A	ACN/10-80	0.9874	0.9859	31.1±3.7	17.3±2.1	0.0870	8	99.2	This Work
5	Benzene	A	ACN/31.3-68.7	0.9957	0.9947	34.5±7.6	19.0±4.3	0.0342	4		This Work
6	Butylbenzene	A	ACN/31.3-68.7	0.9870	0.9999	60.2±1.9	32.7±1.1	0.009	4		This Work
7	Ethylbenzene	A	ACN/31.3-68.7	0.9912	0.9988	47.2±5.1	25.8±2.9	0.0219	4		This Work
8	Isopropylbenzene	A	ACN/31.3-68.7	0.9900	0.9992	52.3±4.4	28.5±2.5	0.0203	4		This Work
9	Anthracene	A	ACN/40-77.5	0.9883	0.9937	61.9±5.4	33.9±3.0	0.0467	6		This Work
10	Phenanthrene	A	ACN/40-77.5	0.9868	0.9949	60.0±4.7	32.9±2.6	0.0405	6		This Work
11	Pyrene	A	ACN/40-77.5	0.9842	0.9962	62.5±4.2	34.0±2.4	0.0366	6		This Work
12	Toluene	A	ACN/31.3-68.7	0.9937	0.9973	40.9±6.6	22.5±3.8	0.0291	4		This Work
13	Anthracene	A	ACN/50-80	0.9893	0.9949	68.0±5.6	37.3±3.1	0.0296	7		Lipford (1985)
14	Naphthalene	A	ACN/50-80	0.9991	0.9894	55.4±6.6	30.5±3.7	0.0347	7	83.3	"
15	1,2-Dihydroxybenzene	G	ACN/10-80	0.9110	0.9927	15.9±1.4	8.88±0.78	0.0319	8		Hanai and Hubert (1983)
16	1,3-Dihydroxybenzene	G	ACN/10-80	0.8964	0.9879	14.0±1.6	7.90±0.89	0.0364	8	95.1	"
17	1,4-Dihydroxybenzene	G	ACN/10-80	0.9103	0.9903	10.1±1.0	5.75±0.57	0.0234	8		"
18	2-Hydroxyacetophenone	G	ACN/10-80	0.8792	0.9821	19.6±2.6	10.9±1.5	0.0620	8	98.5	"
19	4-Methylphenol	G	ACN/10-80	0.9427	0.9976	27.1±1.3	14.9±0.76	0.0310	8		"
20	4-Nitrophenol	G	ACN/10-80	0.9414	0.9965	26.9±3.3	14.9±1.9	0.0372	8		"
21	Phenol	G	ACN/10-80	0.9480	0.9975	20.9±1.0	11.6±0.60	0.0366	8		"
22	2,4-Dinitrophenol	G	ACN/20-80	0.9652	0.9942	31.1±2.7	17.1±1.6	0.0426	7		"
23	2,5-Dinitrophenol	G	ACN/20-80	0.9690	0.9993	31.8±3.0	17.5±1.7	0.0468	7		"
24	2,6-Dinitrophenol	G	ACN/20-80	0.9754	0.9918	29.4±3.1	16.2±1.8	0.0480	7		"
25	2-Bromophenol	G	ACN/20-80	0.9530	0.9952	31.1±2.5	17.1±1.4	0.0388	7		"
26	2-Chlorophenol	G	ACN/20-80	0.9536	0.9949	29.3±2.4	16.1±1.4	0.0378	7		"
27	2-Ethylphenol	G	ACN/20-80	0.9478	0.9943	34.2±3.0	18.8±1.7	0.0464	7		"
28	2-Methylphenol	G	ACN/20-80	0.9604	0.9962	27.7±2.0	15.3±1.1	0.0309	7		"
29	2-Nitrophenol	G	ACN/20-80	0.9700	0.9950	29.2±2.4	15.9±1.4	0.0371	7		"
30	3,4-Dimethylphenol	G	ACN/20-80	0.9466	0.9939	32.2±2.9	17.7±1.7	0.0455	7		"
31	3,4-Dinitrophenol	G	ACN/20-80	0.9441	0.9921	36.3±3.7	20.1±2.1	0.0581	7		"
32	3,5-Dimethylphenol	G	ACN/20-80	0.9493	0.9944	33.2±2.9	18.2±1.6	0.0450	7		"
33	3-Bromophenol	G	ACN/20-80	0.9516	0.9949	34.2±3.8	18.8±1.6	0.0441	7		"
34	3-Chlorophenol	G	ACN/20-80	0.9519	0.9946	32.4±2.7	17.8±1.6	0.0429	7		"
35	3-Ethylphenol	G	ACN/20-80	0.9515	0.9949	33.9±2.8	18.6±1.6	0.0440	7		"

Table 3-1--continued.

Data	Solute	[a]Column	Solvent/% Range	r^2 vs. %	r^2 vs. % $E_T(30)$	Slope(×10^2)	-(y-int)	m	n	b_{ot}	Reference
36	3-Methylphenol	G	ACN/20-80	0.9536	0.9955	27.3±2.1	15.0±1.2	0.0330	7		Hansel and Nubert (1983)
37	3-Nitrophenol	G	ACN/20-80	0.9498	0.9943	27.7±2.4	15.3±1.4	0.0377	7		"
38	4-Bromophanol	G	ACN/20-80	0.9475	0.9942	34.0±3.0	18.7±1.7	0.0466	7		"
39	4-Chlorophenol	G	ACN/20-80	0.9496	0.9949	32.0±2.6	17.6±1.5	0.0411	7		"
40	4-Ethylphenol	G	ACN/20-80	0.9500	0.9946	34.1±2.9	18.7±1.6	0.0451	7		"
41	1-Hydroxynapthalene	G	ACN/30-80	0.9588	0.9877	38.1±5.9	21.0±3.4	0.0582	6	81.3	"
42	2,3,5-Trichlorophenol	G	ACN/30-80	0.9659	0.9915	38.8±5.0	21.2±2.8	0.0489	6		"
43	2,3,5-Trimethyphenol	G	ACN/30-80	0.9640	0.9903	38.9±5.4	21.3±3.0	0.0525	6		"
44	2,3,6-Trimethyphenol	G	ACN/30-80	0.9688	0.9921	38.5±4.5	21.0±2.7	0.0465	6		"
45	2,3-Dichlorophenol	G	ACN/30-80	0.9562	0.9876	38.0±5.9	20.9±3.4	0.0581	6	91.1	"
46	2,3-Dimethylphenol	G	ACN/30-80	0.9641	0.9902	33.2±4.6	18.2±2.6	0.0450	6		"
47	2,4,-Dimethylphenol	G	ACN/30-80	0.9552	0.9842	32.9±5.8	18.0±3.3	0.0568	6	75.6	"
48	2,4,6-Trimethylphenol	G	ACN/30-80	0.9681	0.9922	39.0±4.8	21.3±2.7	0.0473	6		"
49	2,4-Dibromophenol	G	ACN/30-80	0.9606	0.9892	43.2±6.3	23.6±3.5	0.0614	6	84.1	"
50	2,4-Dichlorophenol	G	ACN/30-80	0.9602	0.9891	39.3±5.7	21.6±3.3	0.0563	6	84.4	"
51	2,5-Dichlorophenol	G	ACN/30-80	0.9639	0.9902	39.6±5.5	21.7±3.1	0.0537	6		"
52	2,5-Dimethylphenol	G	ACN/30-80	0.9652	0.9908	33.8±4.5	18.5±2.6	0.0442	6		"
53	2,6-Dibromophenol	G	ACN/30-80	0.9678	0.9919	39.5±4.9	21.6±2.8	0.0485	6		"
54	2,6-Dichlorophenol	G	ACN/30-80	0.9677	0.9918	36.0±4.6	19.7±2.6	0.0447	6		"
55	2,6-Dimethylphenol	G	ACN/30-80	0.9689	0.9921	33.5±4.2	18.3±2.4	0.0407	6		"
56	2-Chloro-5-Methylphenol	G	ACN/30-80	0.9646	0.9905	35.5±4.8	19.5±2.7	0.0475	6		"
57	2-Hydroxynaphthalene	G	ACN/30-80	0.9538	0.9849	36.2±6.2	19.9±3.5	0.0610	6	83.7	"
58	3,4-Dichlorophenol	G	ACN/30-80	0.9589	0.9885	40.6±6.1	22.3±3.5	0.0598	6	85.6	"
59	3,5-Dichlorophenol	G	ACN/30-80	0.9634	0.9904	43.4±5.9	23.7±3.4	0.0582	6		"
60	4-Chloro-2-Methylphenol	G	ACN/30-80	0.9626	0.9890	38.6±5.4	21.2±3.1	0.0534	6	79.8	"
61	4-Chloro-3,5-Dimethylphenol	G	ACN/30-80	0.9588	0.9884	42.4±6.4	23.2±3.6	0.0624	6	85.8	"
62	4-Chloro-3-Methylphenol	G	ACN/30-80	0.9591	0.9881	37.0±5.6	20.3±3.2	0.0553	6	83.0	"
63	4-Hydroxybutylbenzoate	G	ACN/30-80	0.9518	0.9849	45.6±7.8	25.0±4.4	0.0768	6	89.9	"
64	4-Hydroxypropylbenzoate	G	ACN/30-80	0.9481	0.9826	37.9±7.0	20.8±4.0	0.0686	6	90.1	"
65	1-Hydroxy-2,4-Dinitronapthalene	G	ACN/40-80	0.9778	0.9850	44.5±10.1	24.3±5.7	0.0587	5	99.0	"
66	2,3,4,5-Tetrachlorophenol	G	ACN/40-80	0.9759	0.9835	50.9±12.1	27.7±6.8	0.0703	5	98.8	"
67	2,3,4-Trichlorophenol	G	ACN/40-80	0.9732	0.9813	43.1±10.9	23.5±6.1	0.0635	5	98.9	"
68	2,3,5,6-Tetrachlorophenol	G	ACN/40-80	0.9780	0.9852	49.6±11.1	27.0±6.3	0.0649	5	98.7	"
69	2,3,5,6-Tetramethylphenol	G	ACN/40-80	0.9826	0.9894	37.1±7.1	20.2±4.0	0.0413	5	96.7	"
70	2,3,6-Trichlorophenol	G	ACN/40-80	0.9772	0.9844	41.8±9.7	22.8±5.4	0.0562	5	98.7	"
71	2,4,5-Trichlorophenol	G	ACN/40-80	0.9757	0.9830	44.8±10.8	24.5±6.1	0.0629	5	98.7	"
72	2,4,6-Trichlorophenol	G	ACN/40-80	0.9786	0.9855	43.0±9.6	23.5±5.4	0.0558	5	98.6	"
73	3,4,5-Trichlorochenol	G	ACN/40-80	0.9744	0.9821	45.9±11.4	25.1±6.4	0.0662	5	98.8	"
74	4-tert-Butylphenol	G	ACN/40-80	0.9722	0.9800	41.6±10.9	22.7±6.1	0.0635	5	98.6	"
75	Pentachlorophenol	G	ACN/50-80	0.9913	0.9916	48.6±13.6	26.3±7.6	0.0402	4		"

Table 3-1--continued.

Date	Solute	ªColumn	Solvent/% Range	r² vs. Φ	r² vs. E_T(10)	Slope(x10²)	-(y-int)	s	n	b_at	Reference
76	Aniline	G	ACN/10-70	0.9728	0.9951	19.5±1.6	11.1±0.9	0.0308	7		Hanai and Hubert (1985)
77	N-Methylaniline	G	ACN/20-70	0.9874	0.9901	28.0±3.9	15.6±2.2	0.0475	6		"
78	N-Ethylaniline	G	ACN/20-70	0.9870	0.9904	33.0±4.5	18.3±2.6	0.0553	6		"
79	N-Butylaniline	G	ACN/20-70	0.9887	0.9973	53.9±8.6	29.7±4.9	0.0263	4		"
80	N,N-Dimethylaniline	G	ACN/30-70	0.9860	0.9981	38.4±3.0	21.2±1.7	0.0205	5		"
81	N,N-Diethylaniline	G	ACN/40-70	0.9801	0.9970	54.1±9.0	29.7±5.1	0.0276	4		"
82	2-Methylaniline	G	ACN/10-70	0.9704	0.9964	24.5±1.7	13.8±1.0	0.0334	7		"
83	3-Methylaniline	G	ACN/10-70	0.9651	0.9967	25.3±1.7	14.2±1.0	0.0329	7		"
84	4-Methylaniline	G	ACN/10-70	0.9654	0.9973	25.4±1.5	14.3±0.89	0.0301	7		"
85	2,4-Dimethylaniline	G	ACN/20-70	0.9716	0.9978	31.4±21.	17.5±1.2	0.0252	6		"
86	4-Methoxyaniline	G	ACN/10-70	0.9416	0.9981	21.3±1.1	12.2±0.6	0.0212	7		"
87	2,4-Diethoxyaniline	G	ACN/20-70	0.9691	0.9983	36.5±2.1	20.3±1.2	0.0256	6		"
88	2-Chloroaniline	G	ACN/20-70	0.9828	0.9927	30.7±3.7	17.1±2.1	0.0449	6		"
89	3-Chloroaniline	G	ACN/20-70	0.9785	0.9949	32.1±3.2	18.0±1.8	0.0256	6		"
90	4-Chloroaniline	G	ACN/20-70	0.9742	0.9964	31.3±2.6	17.6±1.5	0.0322	6		"
91	2,5-Dichloroaniline	G	ACN/30-70	0.9806	0.9984	44.3±3.3	24.6±1.9	0.0222	5		"
92	3,4-Dichloroaniline	G	ACN/30-70	0.9767	0.9982	43.0±1.4	24.0±1.9	0.0227	5		"
93	4-Bromoaniline	G	ACN/20-70	0.9743	0.9936	33.5±2.8	18.8±1.6	0.0338	6		"
94	2-Nitroaniline	G	ACN/10-70	0.9737	0.9936	28.6±2.6	16.1±1.5	0.0520	7		"
95	3-Nitroaniline	G	ACN/10-70	0.9806	0.9895	26.2±3.1	14.8±1.8	0.0613	7	95.7	"
96	4-Nitroaniline	G	ACN/10-70	0.9770	0.9924	26.3±2.6	14.9±1.5	0.0520	7		"
97	Pyridine	G	ACN/10-70	0.8965	0.9883	17.0±2.1	9.8±1.2	0.0420	7	93.1	"
98	2-Aminopyridine	G	ACN/10-70	0.7384	0.9017	12.5±4.8	7.7±2.8	0.0936	7	90.5	"
99	3-Aminopyridine	G	ACN/10-70	0.8436	0.9661	15.6±3.3	9.3±1.9	0.0661	7	97.1	"
100	2-Methylpyridine	G	ACN/10-70	0.8974	0.9887	22.7±2.8	12.8±1.6	0.0412	7	96.9	"
101	3-Methylpyridine	G	ACN/10-70	0.9142	0.9940	23.5±2.1	13.2±1.2	0.0412	7		"
102	4-Methylpyridine	G	ACN/10-70	0.9072	0.9920	23.6±2.4	13.3±1.4	0.0474	7		"
103	4-Ethylpyridine	G	ACN/20-70	0.9428	0.9973	28.2±2.0	15.7±1.2	0.0250	6		"
104	4-tert-Butylpyridine	G	ACN/30-70	0.9637	0.9956	37.7±4.6	20.8±2.6	0.0309	5		"
105	2,4-Dimethylpyridine	G	ACN/30-70	0.9686	0.9967	26.8±2.8	14.9±1.6	0.0192	5		"
106	2,5-Dimethylpyridine	G	ACN/20-70	0.9432	0.9973	26.6±1.9	14.8±1.1	0.0236	6		"
107	2,6-Dimethylpyridine	G	ACN/10-70	0.9072	0.9920	18.1±4.9	10.5±2.8	0.0556	7		"
108	Pyrazine	G	ACN/10-70	0.7618	0.9196	10.2±3.5	6.2±2.0	0.0681	7	96.3	"
109	2-Methylpyrazine	G	ACN/10-70	0.8009	0.9433	14.2±4.0	8.4±2.3	0.0787	7	98.3	"
110	2,5-Dimethylpyrazine	G	ACN/10-70	0.8185	0.9530	18.2±4.6	10.5±2.7	0.0916	7	99.5	"
111	2,6-Dimethylpyrazine	G	ACN/10-70	0.8103	0.9472	18.1±4.9	10.5±2.8	0.0966	7	97.0	"
112	Quinoline	G	ACN/10-70	0.9378	0.9959	29.8±2.6	16.7±1.5	0.0324	6		"
113	2-Methylquinoline	G	ACN/20-70	0.9425	0.9971	33.6±2.5	18.7±1.4	0.0309	6		"
114	4-Methylquinoline	G	ACN/20-70	0.9573	0.9964	32.4±2.7	18.0±1.5	0.0329	6		"
115	8-Methylquinoline	G	ACN/20-70	0.9551	0.9985	34.7±1.8	19.2±1.1	0.0226	6		"
116	5-Aminoindan	G	ACN/20-70	0.9669	0.9981	34.0±2.0	19.0±1.2	0.0251	6		"
117	5-Aminoindole	G	ACN/10-70	0.9403	0.9968	21.0±1.4	12.2±0.8	0.0274	7		"
118	1-Aminonapthalene	G	ACN/20-70	0.9721	0.9976	36.1±2.5	20.2±1.4	0.0304	6		"
119	2-Aminonapthalene	G	ACN/20-70	0.9691	0.9977	37.1±2.5	20.7±1.4	0.0303	6		"
120	1-Aminoanthracene	G	ACN/30-70	0.9717	0.9976	53.3±4.8	29.5±2.7	0.0326	5		"
121	1-Aminopyrene	G	ACN/40-70	0.9819	0.9937	55.5±13.4	30.7±7.6	0.0401	4		"

Table 3-1--continued.

Date	Solute	aColumn	Solvent/% Range	r² vs. %	r² vs. E_T(30)	Slope(x10²)	-(y-Int)	e	n	b_ot	Reference
122	2,4-Dinitrophenol	H	ACN/20-80	0.9652	0.9917	31.8±3.3	17.7±1.9	0.0521	7		Nenel and Hubert (1983)
123	3-Bromophenol	H	ACN/20-80	0.9495	0.9919	34.7±3.6	19.3±2.1	0.0505	7		"
124	4-Nitrophenol	H	ACN/20-80	0.9536	0.9936	27.3±2.5	15.3±1.4	0.0394	7		"
125	2,3,4,5-Tetrachlorophenol	H	ACN/40-80	0.9675	0.9702	52.4±16.9	26.8±12.5	0.0982	5	71.4	"
126	2,4,5-Trichlorophenol	H	ACN/40-80	0.9688	0.9688	45.7±15.1	25.2±8.5	0.0876	5	70.7	"
127	2,5-Dichlorophenol	H	ACN/30-80	0.9653	0.9877	40.6±6.3	22.5±3.6	0.617	6	61.8	"
128	4-Chloro-3,5-Dimethylphenol	H	ACN/30-80	0.9024	0.9434	42.3±14.4	23.4±8.2	0.1412	6	65.2	"
129	4-Chloro-3-Methylphenol	H	ACN/30-80	0.9587	0.9847	37.9±6.6	21.0±3.7	0.0644	6	68.7	"
130	Toluene	H	ACN/25-40	0.9905	0.9724	28.3±14.5	15.8±8.4	0.0662	4	58.3	Woodburn (1985)
131	n-Butylbenzene	B	ACN/25-40	0.9998	0.9936	47.5±11.6	26.3±6.7	0.0531	4		"
132	1,2,4-Trimethylbenzene	B	ACN/25-50	0.9994	0.9912	38.5±11.0	21.4±6.4	0.0504	4		"
133	Anthracene	B	ACN/25-50	0.9988	0.9917	51.2±14.3	28.5±8.3	0.0653	4		"
134	Benzene	B	ACN/25-50	0.9771	0.9522	22.5±15	12.6±8.9	0.0699	4	53.4	"
135	Biphenyl	B	ACN/25-50	0.9995	0.9919	45.0±12.5	25.0±7.2	0.0567	4		"
136	Bromobenzene	B	ACN/25-50	0.9947	0.9803	31.2±13.5	17.4±7.8	0.0614	4	83.9	"
137	Chlorobenzene	B	ACN/25-50	0.9880	0.9686	29.3±16.1	16.4±9.3	0.0735	4	85.5	"
138	Ethylbenzene	B	ACN/25-50	0.9954	0.9815	34.4±14.4	19.1±8.4	0.0656	4	57.1	"
139	Fluoranthene	B	ACN/25-50	0.9998	0.9968	54.2±9.4	30.2±5.5	0.0429	4		"
140	Fluorobenzene	B	ACN/25-50	0.5846	0.9630	25.2±15.0	14.2±8.7	0.0688	4	78.8	"
141	Iodobenzene	B	ACN/25-50	0.9965	0.9838	34.2±13.4	19.0±7.8	0.0609	4	78.6	"
142	Napthalene	B	ACN/25-50	0.9977	0.9865	36.7±13.1	20.5±7.6	0.0596	5	67.1	"
143	Nitrobenzene	B	ACN/25-50	0.9854	0.9642	23.8±14.0	13.4±8.1	0.0639	4	77.6	"
144	Phenanthrene	B	ACN/25-50	0.9999	0.9950	48.9±10.5	27.2±6.1	0.0484	4		"
145	Pyrene	B	ACN/25-50	0.9999	0.9966	53.9±9.5	30.0±5.5	0.0435	4		"
146	m-Diethylbenzene	B	ACN/25-50	0.9989	0.9895	46.2±14.4	25.6±8.4	0.0661	4	78.1	"
147	n-Propylbenzene	B	ACN/25-50	0.9981	0.9873	40.9±14.1	22.7±8.2	0.0644	4	54.5	"
148	o-Xylene	B	ACN/25-50	0.9941	0.9789	33.0±14.7	18.4±8.5	0.0674	4	55.3	"
149	p-Xylene	B	ACN/25-50	0.9920	0.9752	33.7±16.3	18.7±9.5	0.0747	4	79.8	"
150	Chrysene	B	ACN/30-60	0.9997	0.9981	63.3±34.4	35.2±19.8	0.0378	3		"
151	1,2,4-Trimethylbenzene	C	ACN/30-60	0.9692	0.9993	41.6±3.0	22.9±1.7	0.0147	4		"
152	Anthracene	C	ACN/30-60	0.9881	0.9994	50.7±3.7	28.0±2.1	0.0174	4		"
153	Benzene	C	ACN/30-60	0.9889	0.9936	26.9±6.6	15.0±3.8	0.0298	4		"
154	Biphenyl	C	ACN/30-60	0.9908	0.9995	46.8±3.5	25.9±x.0	0.0147	4		"
155	Bromobenzene	C	ACN/30-60	0.9962	0.9973	34.7±5.4	19.3±3.1	0.0247	4		"
156	Chlorobenzene	C	ACN/30-60	0.9976	0.9964	33.0±6.1	18.3±3.5	0.0275	4		"
157	Chrysene	C	ACN/30-60.	0.9823	0.9985	60.7±7.1	33.5±4.0	0.0325	4		"
158	Ethylbenzene	C	ACN/30-60	0.9966	0.9977	37.8±5.5	20.9±3.2	0.0250	4		"
159	Fluoranthene	C	ACN/30-60	0.9869	0.9994	53.8±4.1	29.7±2.4	0.0175	4		"
160	Fluorobenzene	C	ACN/30-60	0.9982	0.9954	29.0±6.1	16.2±3.5	0.0272	4		"
161	Iodobenzene	C	ACN/30-60	0.9946	0.9986	37.1±4.2	20.5±2.4	0.0189	4		"

Table 3-1--continued.

Data	Solute	ᵃColumn	Solvent/% Range	r² vs. %	r² vs. E_T(30)	Slope(x10²)	-ly-Int	s	n	b_αt	Reference
162	Napthalene	C	ACN/30-60	0.9936	0.9988	39.1±4.0	21.7±2.3	0.0185	4		Woodburn (1985)
163	Nitrobenzene	C	ACN/30-60	0.9905	0.9952	27.1±5.7	15.2±3.2	0.0259	4		"
164	p-Xylene	C	ACN/30-60	0.9946	0.9903	37.6±4.6	20.8±2.6	0.0214	4		"
165	Phenanthrene	C	ACN/30-60	0.9867	0.9993	50.2±4.1	27.8±2.4	0.0180	4		"
166	Pyrene	C	ACN/30-60	0.9848	0.9990	54.0±5.3	29.8±3.1	0.0238	4		"
167	Toluene	C	ACN/30-60	0.9983	0.9955	32.0±6.4	17.7±3.7	0.0295	4		"
168	m-Diethylbenzene	C	ACN/30-60	0.9915	0.9998	47.9±2.2	26.4±1.3	0.0090	4		"
169	n-Butylbenzene	C	ACN/30-60	0.9920	0.9994	50.6±3.6	27.9±2.1	0.0169	4		"
170	n-Propylbenzene	C	ACN/30-60	0.9945	0.9989	44.0±4.5	24.2±2.6	0.0202	4		"
171	o-Xylene	C	ACN/30-60	0.9949	0.9984	36.9±4.6	20.4±2.6	0.0204	4		"
172	n-Hexylbenzene	C	ACN/30-60	0.9976	0.9991	60.8±23.2	33.3±13.2	0.0199	3		"
173	1,2,4-Trimethylbenzene	D	ACN/30-80	0.9786	0.9973	49.5±3.6	27.2±2.0	0.0371	6	65.7	"
174	Benzene	D	ACN/30-80	0.9877	0.9883	34.1±5.2	19.0±2.9	0.0535	6		"
175	Biphenyl	D	ACN/30-80	0.9739	0.9905	54.3±12.9	29.9±1.7	0.0302	6		"
176	Bromobenzene	D	ACN/30-80	0.9018	0.9948	42.0±4.2	23.2±2.4	0.0439	6		"
177	Chlorobenzene	D	ACN/30-80	0.9813	0.9951	40.7±4.0	22.6±2.2	0.0413	6		"
178	Ethylbenzene	D	ACN/30-80	0.9813	0.9945	45.6±4.7	25.2±2.7	0.0492	6		"
179	Fluorobenzene	D	ACN/30-80	0.9841	0.9913	36.2±4.7	20.2±2.7	0.0491	6		"
180	Iodobenzene	D	ACN/30-80	0.9825	0.9952	44.5±4.3	24.6±2.4	0.0446	6		"
181	Nitrobenzene	D	ACN/30-80	0.9843	0.9915	35.1±4.6	19.6±2.6	0.0470	6		"
182	n-Propylbenzene	D	ACN/30-80	0.9796	0.9966	51.9±4.2	28.5±2.4	0.0438	6		"
183	Toluene	D	ACN/30-80	0.9846	0.9914	39.7±5.1	22.0±2.9	0.0534	6		"
184	m-Diethylbenzene	D	ACN/30-80	0.9791	0.9976	56.0±3.9	30.7±2.2	0.0396	6		"
185	o-Xylene	D	ACN/30-80	0.9841	0.9947	44.6±4.6	24.6±2.6	0.0471	6		"
186	p-Xylene	D	ACN/30-80	0.9816	0.9960	45.3±4.0	25.0±2.2	0.0415	6		"
187	Anthracene	D	ACN/40-80	0.9807	0.9981	58.9±4.8	32.4±2.7	0.0281	5		"
188	Chrysene .	D	ACN/40-80	0.9758	0.9995	67.5±2.9	37.0±1.6	0.0164	5		"
189	Fluoranthene	D	ACN/40-80	0.9761	0.9989	62.1±3.6	34.1±2.0	0.0220	5		"
190	Napthalene	D	ACN/40-80	0.9792	0.9962	48.5±5.5	26.8±3.1	0.0324	5		"
191	Phenanthrene	D	ACN/40-80	0.9774	0.9990	58.6±3.3	32.2±1.9	0.0199	5		"
192	Pyrene	D	ACN/40-80	0.9750	0.9992	61.0±3.1	33.5±1.7	0.0182	5		"
193	n-Butylbenzene	D	ACN/40-80	0.9780	0.9985	59.8±4.3	32.8±2.4	0.0253	5		"
194	n-Hexylbenzene	D	ACN/40-80	0.9764	0.9988	70.5±4.6	38.5±2.6	0.0268	5		"
195	Bromobenzene	E	ACN/40-70	0.9976	0.9969	45.9±7.7	25.0±4.3	0.0149	4		"
196	Fluorobenzene	E	ACN/40-70	0.9981	0.9963	40.7±7.5	22.5±4.2	0.0145	4		"
197	Iodobenzene	E	ACN/40-70	0.9968	0.9977	47.8±7.0	26.2±3.9	0.0134	4		"
198	Nitrobenzene	E	ACN/40-70	0.9984	0.9958	39.5±7.9	22.0±4.4	0.0149	4		"
199	1,2,4-Trimethylbenzene	E	ACN/50-70	0.9966	0.9978	52.2±7.3	28.5±4.1	0.0143	4		"
200	Anthracene	E	ACN/50-70	0.9957	0.9983	58.5±7.4	31.9±4.1	0.0142	4		"
201	Benzene	E	ACN/50-70	0.9995	0.9931	38.2±9.7	21.1±5.5	0.0187	4		"

Table 3-1--continued.

Date	Solute	a Column	Solvent/% Range	r² vs. %	r² vs. E_T(30)	Slope(x10²)	-(y-int)	e	n	b a_t	Reference
202	Biphenyl	E	ACN/50-70	0.9963	0.9980	55.1±7.5	30.2±4.2	0.0146	4		Woodburn (1985)
203	Chrysene	E	ACN/50-70	0.9947	0.9908	65.9±6.8	35.7±3.8	0.0131	4		"
204	Ethylbenzene	E	ACN/50-70	0.9967	0.9976	48.9±7.2	26.8±4.1	0.0141	4		"
205	Napthalene	E	ACN/50-70	0.9968	0.9974	49.0±7.5	26.9±4.2	0.0146	4		"
206	Phenanthrene	E	ACN/50-70	0.9951	0.9989	57.6±6.2	31.4±3.5	0.0116	4		"
207	Pyrene	E	ACN/50-70	0.9953	0.9986	60.0±6.8	32.4±3.8	0.0129	4		"
208	Toluene	E	ACN/50-70	0.9967	0.9975	43.4±6.5	23.9±3.7	0.0127	4		"
209	m-Diethylbenzene	E	ACN/50-70	0.9967	0.9977	57.7±8.5	31.4±4.8	0.0161	4		"
210	n-Butylbenzene	E	ACN/50-70	0.9968	0.9975	59.0±8.8	32.1±5.0	0.0172	4		"
211	n-Hexylbenzene	E	ACN/50-70	0.9961	0.9982	69.0±9.1	37.3±5.1	0.0171	4		"
212	n-Propylbenzene	E	ACN/50-70	0.9962	0.9980	54.2±7.4	29.6±4.1	0.0142	4		"
213	o-Xylene	E	ACN/50-70	0.9970	0.9976	47.8±7.2	26.2±4.0	0.0137	4		"
214	p-Xylene	E	ACN/50-70	0.9966	0.9979	48.5±6.8	26.6±3.8	0.0131	4		"
215	Acetophenone	F	ACN/50-80	0.9989	0.9960	33.7±6.5	19.3±3.6	0.0192	4		Jandera (1985)
216	Anisole	F	ACN/50-80	0.9965	0.9801	40.6±13.6	23.0±7.6	0.0401	4	29.3	"
217	Benzaldehyde	F	ACN/50-80	0.9985	0.9947	38.6±8.6	22.0±4.8	0.0252	4		"
218	Benzonitrile	F	ACN/50-80	.9983	0.9920	39.2±10.7	22.3±6.0	0.0317	4		"
219	Benzophenone	F	ACN/50-80	.9997	0.9976	47.9±7.1	26.9±4.0	0.0210	4		"
220	Benzotrichloride	F	ACN/50-80	0.9984	0.9989	52.4±5.4	29.2±3.0	0.0158	4		"
221	Bromobenzene	F	ACN/50-80	0.9993	0.9942	44.4±10.3	24.9±5.7	0.0303	4		"
222	Chlorobenzene	F	ACN/50-80	0.9998	0.9969	44.6±7.6	25.1±4.3	0.0225	4		"
223	Chlorobromuron	F	ACN/50-80	0.9998	0.9966	46.6±8.3	26.2±4.6	0.0245	4		"
224	Di-n-Butylether	F	ACN/50-80	0.9975	0.9947	46.6±10.3	25.9±5.8	0.0304	4		"
225	Ethyl benzoate	F	ACN/50-80	0.9990	0.9934	45.4±11.3	25.5±6.3	0.0333	4		"
226	Linuron	F	ACN/50-80	0.9999	0.9970	46.6±7.8	26.3±4.4	0.0231	4		"
227	Methyl benzoate	F	ACN/50-80	0.9976	0.9926	39.0±10.3	22.1±5.7	0.0303	4		"
228	Nitrobenzene	F	ACN/50-80	0.9995	0.9948	40.0±8.8	22.7±4.9	0.0261	4		"
229	Phenetole	F	ACN/50-80	0.9892	0.9765	41.9±19.8	23.6±11.0	0.0584	4	29.3	"
230	Phenol	F	ACN/50-80	0.9587	0.9691	43.4±2.4	25.0±13.2	0.0697	4	54.0	"
231	Phenyl acetate	F	ACN/50-80	0.9969	0.9911	36.0±10.4	20.5±5.8	0.0306	4		"
232	Styrene	F	ACN/50-80	0.9988	0.9944	45.5±10.4	25.5±5.8	0.0307	4		"
233	m-Cresol	F	ACN/50-80	0.9937	0.9833	39.4±15.6	22.6±8.7	0.0462	4	29.3	"
234	n-Butylbromide	F	ACN/50-80	0.9990	0.9966	44.8±8.0	25.1±4.5	0.0235	4		"
235	n-Butylphenyl Carbamate	F	ACN/50-80	0.9999	0.9981	48.0±6.3	27.0±3.5	0.0186	4		"
236	n-Heptane	F	ACN/50-80	0.9990	0.9986	54.5±6.1	29.9±3.4	0.0181	4		"
237	n-Octane	F	ACN/50-80	0.9981	0.9976	59.0±8.8	32.3±4.9	0.0260	4		"
238	n-Propylphenylether	F	ACN/50-80	0.9999	0.9960	46.1±8.9	25.9±5.0	0.0262	4		"
239	o-Cresol	F	ACN/50-80	0.9952	0.9959	39.0±7.7	22.4±4.3	0.0226	4		"
240	p-Cresol	F	ACN/50-80	0.9984	0.9900	41.9±5.6	23.9±3.2	0.0166	4		"
241	1,2,4-Trimethylbenzene	B	MeOH/35-60	0.9930	0.9852	53.3±11.9	30.5±11.7	0.0699	4	54.5	Woodburn (1985)

84

Table 3-1--continued.

Data	Solute	Column[a]	Solvent/% Range	r^2 vs. %	r^2 vs. $E_T(30)$	Slope(×10²)	-(y-int)	e	n	b_{nt}	Reference
242	Anthracene	B	MeOH/35-60	0.9976	0.9923	70.7±18.9	40.4±11.1	0.0665	4	83.8	Woodburn (1985)
243	Benzene	B	MeOH/35-60	0.9606	0.9535	33.1±22.2	19.3±13.0	0.0701	4	83.6	"
244	Biphenyl	B	MeOH/35-60	0.9959	0.9895	61.6±19.3	35.2±11.3	0.0600	4	80.3	"
245	Bromobenzene	B	MeOH/35-60	0.9843	0.9866	45.1±22.7	26.0±13.3	0.0799	4	83.7	"
246	Chlorobenzene	B	MeOH/35-60	0.9837	0.9723	42.2±21.6	24.2±12.7	0.0762	4	76.5	"
247	Chrysene	B	MeOH/35-60	0.9994	0.9966	92.6±69.1	52.8±40.5	0.0587	3		"
248	Ethylbenzene	B	MeOH/35-60	0.9867	0.9763	46.1±21.8	26.5±12.8	0.0767	4	61.8	"
249	Fluorobenzene	B	MeOH/35-60	0.9766	0.9633	36.1±21.4	21.0±12.6	0.0753	4	83.2	"
250	Iodobenzene	B	MeOH/35-60	0.9095	0.9802	48.3±20.9	27.8±12.3	0.0734	4	82.3	"
251	Napthalene	B	MeOH/35-60	0.9910	0.9823	51.9±21.2	29.8±12.4	0.0745	4	79.5	"
252	Nitrobenzene	B	MeOH/35-60	0.9598	0.9629	36.2±21.6	21.2±12.7	0.0760	4	84.7	"
253	Phenanthrene	B	MeOH/35-60	0.9977	0.9926	69.2±18.2	39.6±10.7	0.0640	4		"
254	Pyrene	B	MeOH/35-60	0.9987	0.9945	77.8±17.7	44.4±10.4	0.0619	4		"
255	Toluene	B	MeOH/35-60	0.9797	0.9672	39.2±21.9	22.7±12.9	0.0772	4	64.3	"
256	m-Diethylbenzene	B	MeOH/35-60	0.9961	0.9899	60.7±18.6	34.5±10.9	0.0654	5	54.	"
257	n-Butylbenzene	B	MeOH/35-60	0.9959	0.9895	65.1±20.5	37.0±12.0	0.0718	4	63.6	"
258	n-Propylbenzene	B	MeOH/35-60	0.9920	0.9837	54.6±21.4	31.2±12.6	0.0753	4	61.8	"
259	o-Xylene	B	MeOH/35-60	0.9868	0.9765	45.4±21.4	26.1±12.6	0.0754	4	57.1	"
260	p-Xylene	B	MeOH/35-60	0.9863	0.9756	47.0±22.6	27.0±13.3	0.0795	4	88.4	"
261	1,2,4-Trimethylbenzene	C	MeOH/40-75	0.9999	0.9958	60.6±7.2	34.3±4.2	0.0457	5		"
262	Benzene	C	MeOH/40-75	0.9960	0.9853	39.5±8.9	22.6±5.1	0.0557	5	67.4	"
263	Bromobenzene	C	MeOH/40-75	0.9986	0.9905	51.0±9.2	29.1±5.3	0.0578	5		"
264	Chlorobenzene	C	MeOH/40-75	0.9988	0.9913	48.9±8.4	27.9±4.9	0.0529	5		"
265	Ethylbenzene	C	MeOH/40-75	0.9997	0.9924	54.8±7.7	31.1±4.5	0.0438	5		"
266	Fluorobenzene	C	MeOH/40-75	0.9973	0.9872	43.0±9.0	24.6±5.2	0.0564	5	88.9	"
267	Iodobenzene	C	MeOH/40-75	0.9998	0.9950	55.0±7.2	31.3±4.2	0.0451	5		"
268	Napthalene	C	MeOH/40-75	0.9999	0.9897	54.7±10.3	31.0±5.9	0.0644	4	66.6	"
269	Nitrobenzene	C	MeOH/40-75	0.9983	0.9903	41.8±7.6	24.0±4.4	0.0477	5		"
270	Toluene	C	MeOH/40-75	0.9986	0.9910	46.7±8.2	26.6±4.7	0.0514	5		"
271	p-Xylene	C	MeOH/40-75	0.9994	0.9933	54.2±8.2	30.8±4.7	0.0514	5		"
272	Anthracene	C	MeOH/50-75	0.9999	0.9898	75.3±23.3	42.5±13.4	0.0681	4	56.1	"
273	Biphenyl	C	MeOH/50-75	0.9999.	0.9887	68.7±22.4	38.9±12.9	0.0655	4	53.7	"
274	Chrysene	C	MeOH/50-75	0.9993	0.9913	89.8±25.7	50.6±14.8	0.0751	4		"
275	Phenanthrene	C	MeOH/50-75	0.9996	0.9897	73.9±23.0	41.8±13.2	0.0671	4	53.4	"
276	Pyrene	C	MeOH/50-75	0.9883	0.9906	80.1±23.7	45.3±13.6	0.0696	4		"
277	m-Diethylbenzene	C	MeOH/50-75	0.9998	0.9976	74.8±11.3	42.2±6.5	0.0329	4		"
278	n-Butylbenzene	C	MeOH/50-75	0.9999	0.9990	77.8±7.5	43.9±4.3	0.0220	4		"
279	n-Propylbenzene	C	MeOH/50-75	0.9999	0.9983	68.4±8.5	38.7±4.9	0.0248	4		"
280	p-Xylene	C	MeOH/50-75	0.9999	0.9980	56.6±7.8	32.1±4.5	0.0228	4		"
281	o-Xylene	C	MeOH/50-80	0.9999	0.9976	61.2±9.2	34.5±5.3	0.0303	4		"

Table 3-1--continued.

Data	Solute	^{a}Column	Solvent/% Range	r^2 vs. %	r^2 vs. $E_T(30)$	Slope($\times 10^2$)	$-(y\text{-int})$	e	n	b_{dt}	Reference
282	Toluene	C	MeOH/50-75	0.9906	0.9966	55.4±9.8	31.4±5.6	0.0325	4		Woodburn (1985)
283	n-Hexylbenzene	C	MeOH/60-75	0.9998	1.000	111.8±5.6	57.2±6.4	0.005	3		"
284	1,2,4-Trimethylbenzene	D	MeOH/50-80	0.9997	0.9901	69.0±9.1	38.8±5.2	0.0301	4		"
285	Anthracene	D	MeOH/50-80	0.9984	0.9996	82.4±5.1	46.3±2.9	0.0163	4		"
286	Benzene	D	MeOH/50-80	0.9999	0.9955	47.2±9.5	26.9±5.5	0.0317	4		"
287	Biphenyl	D	MeOH/50-80	0.9987	0.9993	76.5±6.0	43.1±3.5	0.0207	4		"
288	Bromobenzene	D	MeOH/50-80	0.9998	0.9979	59.1±8.4	33.5±4.8	0.0274	4		"
289	Chlorobenzene	D	MeOH/50-80	0.9999	0.9968	57.1±9.8	32.4±5.6	0.0327	4		"
290	Ethylbenzene	D	MeOH/50-80	0.9999	0.9971	64.0±1.1	36.2±6.1	0.0346	4		"
291	Fluorobenzene	D	MeOH/50-80	0.9999	0.9955	50.8±10.5	29.0±6.0	0.0342	4		"
292	Iodobenzene	D	MeOH/50-80	0.9998	0.9978	62.5±9.1	35.3±5.2	0.0294	4		"
293	Napthalene	D	MeOH/50-80	0.9994	0.9907	64.4±6.8	36.4±3.9	0.0230	4		"
294	Nitrobenzene	D	MeOH/50-80	0.9999	0.9963	47.2±8.7	27.0±5.0	0.0287	4		"
295	Phenanthrene	D	MeOH/50-80	0.9983	0.9996	80.7±4.7	45.4±2.7	0.0161	4		"
296	Pyrene	D	MeOH/50-80	0.9973	0.9999	87.3±2.6	48.9±1.5	0.008	4		"
297	m-Diethylbenzene	D	MeOH/50-80	0.9995	0.9986	79.5±9.1	44.6±5.2	0.0297	4		"
298	n-Butylbenzene	D	MeOH/50-80	0.9990	0.9992	82.6±7.4	46.3±4.2	0.0235	4		"
299	n-Propylbenzene	D	MeOH/50-80	0.9996	0.9983	73.3±9.2	41.2±5.3	0.0300	4		"
300	p-Xylene	D	MeOH/50-80	0.9999	0.9972	62.8±10.2	35.4±5.9	0.0334	4		"
301	Chrysene	D	MeOH/60-80	0.9992	0.9999	98.5±13.5	55.1±7.6	0.007	3		"
302	n-Hexylbenzene	D	MeOH/60-80	0.9997	0.9996	28.5±14.1	58.9±16.2	0.0180	3		"
303	Acetophenone	F	MeOH/60-90	0.9990	0.9989	50.5±5.0	29.2±2.8	0.0136	4		Jendere (1985)
304	Anisole	F	MeOH/60-90	0.9979	0.9989	57.0±17.5	32.8±9.9	0.0473	4		"
305	Benzaldehyde	F	MeOH/60-90	0.9911	0.9777	55.1±25.3	31.9±14.4	0.0685	4	60.9	"
306	Benzonitrile	F	MeOH/60-90	0.9992	0.9952	53.6±11.3	31.0±6.4	0.0305	4		"
307	Benzophenone	F	MeOH/60-90	0.9996	0.9980	69.4±9.5	39.6±5.4	0.0257	4		"
308	Benzotrichloride	F	MeOH/60-90	0.9988	0.9988	79.8±8.3	45.2±4.7	0.0225	4		"
309	Bromobenzene	F	MeOH/60-90	0.9999	0.9951	67.1±14.3	38.2±8.1	0.0387	4		"
310	Chlorobenzene	F	MeOH/60-90	0.9998	0.9944	64.7±14.7	36.9±8.3	0.0397	4		"
311	Chlorobromuron	F	MeOH/60-90	0.9978	0.9922	73.9±19.9	42.2±11.3	0.0538	4		"
312	Di-n-Butylether	F	MeOH/60-90	0.9988	0.9990	74.5±7.3	42.3±4.1	0.0195	4		"
313	Ethyl benzoate	F	MeOH/60-90	0.9996	0.9978	68.2±9.7	38.9±5.5	0.0263	4		"
314	Linuron	F	MeOH/60-90	0.9993	0.9985	70.1±8.4	40.0±4.7	0.0227	4		"
315	m-Cresol	F	MeOH/60-90	0.9917	0.9772	62.6±29.1	36.2±16.5	0.0786	4	50.5	"
316	Methyl Benzoate	F	MeOH/60-90	0.9996	0.9981	59.4±7.9	34.1±4.5	0.0213	4		"
317	n-Butyl Bromide	F	MeOH/60-90	0.9990	0.9927	66.9±17.5	38.1±9.9	0.0472	4		"
318	n-Heptane	F	MeOH/60-90	0.9999	0.9962	95.1±17.8	53.5±10.1	0.0478	4		"
319	Nitrobenzene	F	MeOH/60-90	0.9944	0.9841	58.2±22.5	33.5±12.7	0.0608	4	61.1	"
320	o-Cresol	F	MeOH/60-90	0.9896	0.9792	61.0±27.0	35.3±15.3	0.0730	4	67.9	"
321	p-Cresol	F	MeOH/60-90	0.9984	0.9848	58.8±22.2	34.0±12.6	0.0600	4	81.1	"

Table 3-1--continued.

Data	Solute	aColumn	Solvent/% Range	r² vs. %	r² vs. %, $E_T(30)$	Slope(x10²)	-(y-int)	a	n	bot	Reference
322	Phenetole	F	MeOH/60-90	0.9992	0.9971	61.3±10.1	35.1±5.7	0.0274	4		Jandera (1985)
323	Phenol	F	MeOH/60-90	0.9460	0.9198	61.4±55.1	35.7±28.6	0.1403	4	62.5	"
324	Phenyl acetate	F	MeOH/60-90	0.9994	0.9905	50.1±6.0	29.0±3.4	0.0161	4		"
325	Styrene	F	MeOH/60-90	0.9999	0.9961	65.7±12.6	37.5±7.1	0.0340	4		"
326	n-Butylphenylcarbamate	F	MeOH/60-90	0.9995	0.9926	75.3±19.8	43.0±11.2	0.0535	4		"
327	n-Propyl phenyl ether	F	MeOH/60-90	0.9999	0.9957	68.0±13.6	4.0±7.7	0.0369	4		"
328	Benzene	A	MeOH/48.9-70.7	0.9953	0.9948	38.2±8.3	21.4±4.7	0.0304	4		This Work
329	Butylbenzene	A	MeOH/48.9-70.7	0.9980	0.9900	73.7±22.5	40.8±12.8	0.0816	4		This Work
330	Isopropylbenzene	A	MeOH/48.9-70.7	0.9981	0.9904	63.0±18.8	35.0±10.7	0.0683	4		This Work
331	Toluene	A	MeOH/48.9-70.7	0.9972	0.9922	47.4±12.8	26.5±7.3	0.0465	4		This Work
332	Ethylbenzene	A	MeOH/48.9-70.7	0.9981	0.9911	55.8±16.1	31.0±9.2	0.0584	4		This Work

aColumns

A. 15 cm X 4.6 mm, 5 μm Ultrasphere ODS (Altex)

B. 5 cm X 4.6 mm, 10 μm Sepralyte C2 (Analytichem)

C. 5 cm X 4.6 mm, 10 μm Sepralyte C4 (Analytichem)

D. 5 cm X 4.6 mm, 10 μm Sepralyte C8 (Analytichem)

E. 5 cm X 4.6 mm, 10 μm Sepralyte C18 (Analytichem)

F. 30 cm X 3.8 mm, 7.5 μm Silasorb C8 (Lachema)

G. 15 cm X 4.1 mm, 5 μm Unisil Q C18 (Gasukuro Kogyo)

H. 15 cm X 4.1 mm, 5 μm Hypersil ODS (Shandon Southern)

bot is the significance level for the F ratio (n-2, n-3 degrees of freedom) for fitting the the data to either a linear or quadratic model.

latter comparison. The type of column is denoted by a single letter (A-G); a key appears at the end of the table. Confidence intervals reported for the log k' versus $E_T(30)$ comparisons are for the 95% level of confidence (t statistic, n-2 degrees of freedom). The column in Table 3-1 labeled "α%" is the significance level of the F-ratio for fitting the data to either a linear or quadratic model (discussed below). The last column in Table 3-1 denotes the source of the retention data.

Weighted least squares regression calculations were not used in these correlations for two primary reasons. First, it is difficult to estimate the relative uncertainty in each individual log k' value. There are two main sources of error in log k'; one of these is systematic and the other is random. The systematic error may be caused by the method used to evaluate the void volume of the column. There are many ways to estimate this, and two recent papers have dealt with the problems inherent in this type of measurement (Knox and Kaliszan, 1985; Zhu, 1985). Any systematic error in t_o will lead to an error in the calculated log k' value. This is especially the case where retention times are small, and thus k' approaches zero and log k' becomes very large and negative.

Moreover, in addition to systematic errors in the measurement of t_o and t_r, there will always be a certain amount of random variability in the measured t_o, and hence

an extra source of error is introduced. This is especially a problem for polar solutes which are poorly retained and/or at high concentrations of organic modifier. For example, when t_0 is 1.2 minutes with an uncertainty of ±0.05 minutes, a peak with t_r = 3.0 minutes will have a κ' of 1.5 and log k of 0.176±0.04, which is 24% on a relative basis. This error is in addition to any that may be caused by systematic t_0 measurement errors. While there is uncertainty in the calculated log k' values, these errors are also present in all chromatographic retention data and will thus affect both types of comparisons presented here.

Secondly, in many cases, k' values reported in the literature have been calculated using void volume measurement techniques that are not specified. Thus, it is simply not possible to estimate the relative variance of log k' values for these cases. Even if the variance were determined by multiple injections at each concentration, the effect of systematic errors in the evaluation of void volume is still not represented, and there is at present no absolutely infallible way of measuring the true void volume seen by the solute. It is probable that each log k' value will have an uncertainty of at least ±0.1 log units, though the variation in uncertainty as a function of organic modifier concentration cannot be estimated accurately.

Overall, the average r^2 value for plotting the data versus percent organic modifier is 0.9783 and versus $E_T(30)$

polarity is 0.9910. However, these averages fail to convey a sense of how the values are distributed among the 332 data sets. In Figures 3-7 and 3-8, histograms are shown for each of the two methods of plotting the retention data. It can be seen that a much wider range in r^2 values is encompassed when the data are plotted with respect to percent organic modifier. However, the x-axis of the two histograms is scaled differently in each case. So in Figures 3-9 and 3-10, histograms have been constructed for a standardized range (0.95 to 1.00), and thus the distribution is more clearly presented. By confining the histogram to those r^2 values greater than 0.95, 89.2 and 98.2% of the sets of "versus percent organic" and "versus $E_T(30)$," respectively, are represented. Figures 3-9 and 3-10 clearly demonstrate that the distribution is far less skewed among the "log k' versus $E_T(30)$" sets, and a much higher number of sets have r^2 values of greater than 0.9950. Squared correlation coefficients (r^2) are reported for two reasons. First, it is a more conservative measure of correlation, and secondly, r^2 represents the fraction of the variance in log k' values that is accounted for by a linear statistical model (Edwards, 1984).

A poor r^2 value (defined here as one less than 0.9900) may be due to either a large amount of symmetrical scatter around the line and/or systematic deviation (curvature).
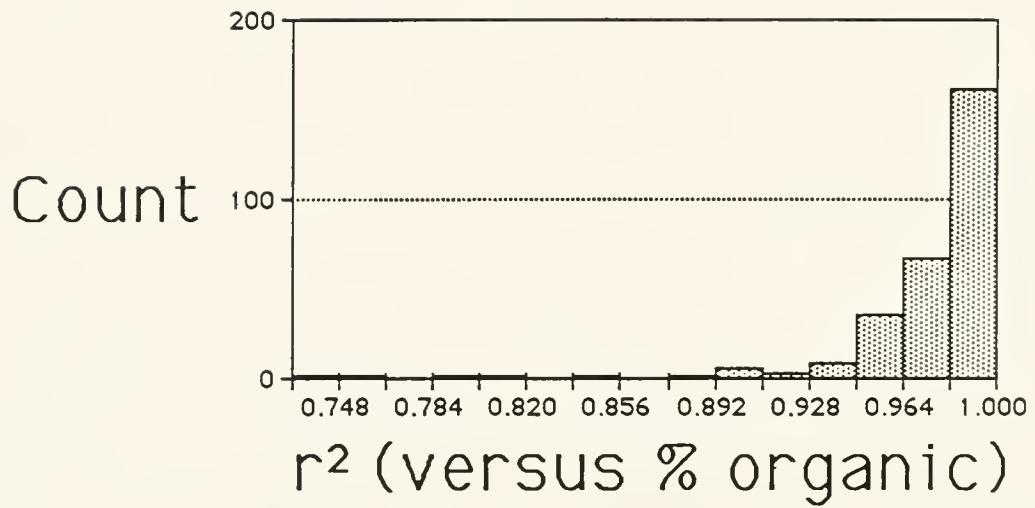
Figure 3-7. Histogram of r^2 values for the 332 retention data sets plotted with respect to percent organic modifier.
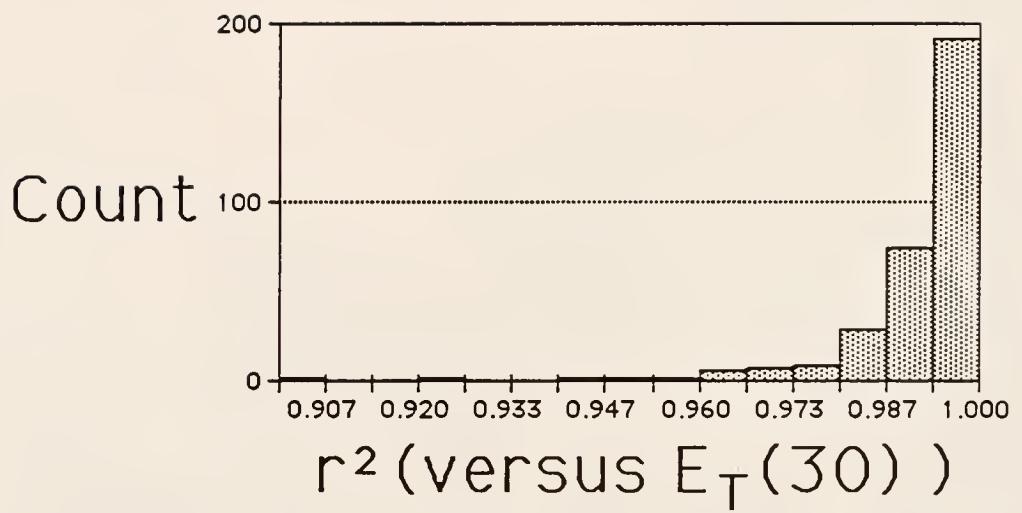
Figure 3-8. Histogram of r^2 values for the 332 retention data sets plotted with respect to $E_T(30)$ polarity.
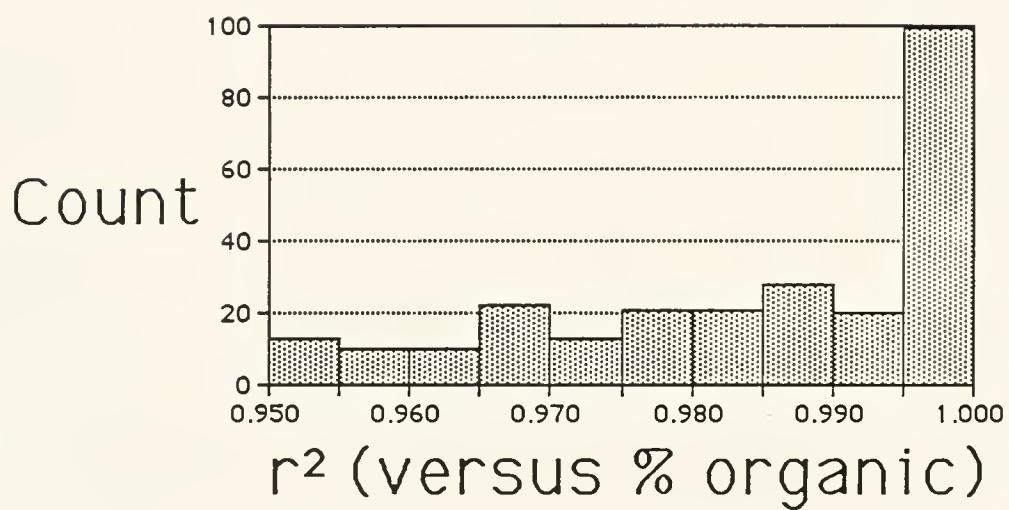
Figure 3-9. Modified histogram of r^2 values for the 332 retention data sets plotted with respect to percent organic modifier. Range of r^2 values limited to 0.95 to 1.00.
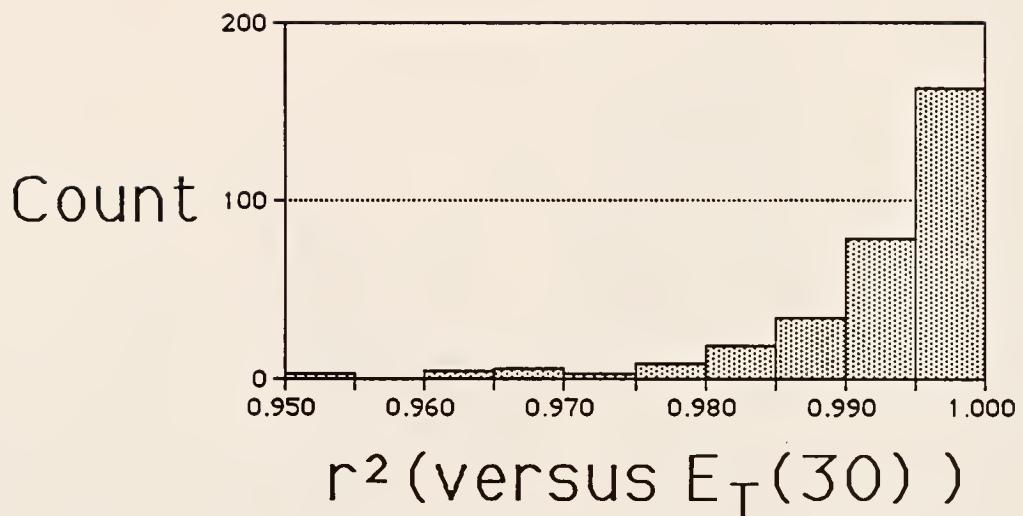
Figure 3-10. Modified histogram of r^2 values for the 332
retention data sets plotted with respect to
$E_T(30)$ polarity. Range of r^2 values limited
to 0.95 to 1.00.

When comparing the relative fit of a linear or quadratic model, the ratio of the variance (s^2) for the two models is known as an F-value and can be used to assess whether the "fit" is significantly better with a particular model. For each case in which the r^2 value for the log k' versus $E_T(30)$ polarity linear regression fell below 0.9900, an F value was calculated and the significance level ($\alpha\%$) was determined for fitting the data to a quadratic model (2nd-degree polynomial). The quadratic model did not fit the data significantly better (at the 90% confidence level) except in 16 out of the 332 sets; these were primarily the tri- and tetrachlorophenols found in the data of Hanai and Hubert (1983).

In Table 3-2, the average squared correlation coefficients for the various columns and mobile phases are summarized. Since the distribution of the r^2 values is highly skewed, both median and mean values are reported in order to present a more accurate "picture" of the data. Also, a Δr^2 value is reported for each LC column and represents the difference between the mean r^2 values for the two types of correlations discussed herein. Thus, a positive value denotes a better correlation versus $E_T(30)$ polarity values. The largest difference between the two methods of treatment is for Column G (Hypersil ODS; from Hanai and Hubert, 1983) in acetonitrile/water mixtures, in

Table 3-2.
Mean and median r² values for correlations shown in Table 3-1.

r^2 values
(MEAN/MEDIAN/STD. DEV.)

Column/Mobile Phase	n	VS. % OM	VS. $E_T(30)$ POLARITY	Δr^2
A/ACN (C-18)	14	0.9890/0.9879/0.0048	0.9945/0.9947/0.0038	0.0055
G/ACN (C-18)	117	0.9531/0.9646/0.0438	0.9897/0.9933/0.0137	0.0366
H/ACN (C-18)	8	0.9539/0.9619/0.0219	0.9790/0.9862/0.0163	0.0251
B/ACN (C-2)	21	0.9948/0.9977/0.0063	0.9828/0.9865/0.0128	-0.0120
C/ACN (C-4)	22	0.9921/0.9946/0.0070	0.9980/0.9987/0.0017	0.0059
D/ACN (C-8)	22	0.9802/0.9801/0.0036	0.9960/0.9964/0.0031	0.0158
E/ACN (C-18)	20	0.9967/0.9967/0.0011	0.9975/0.9977/0.0013	0.0008
F/ACN (C-8)	26	0.9965/0.9987/0.0081	0.9932/0.9954/0.0070	-0.0033
B/MeOH (C-2)	20	0.9880/0.9903/0.0105	0.9805/0.9830/0.0119	-0.0075
C/MeOH (C-4)	21	0.9987/0.9997/0.0025	0.9930/0.9913/0.0041	-0.0057
D/MeOH (C-8)	21	0.9989/0.9997/0.0024	0.9982/0.9983/0.0014	-0.0007
F/MeOH (C-8)	25	0.9960/0.9992/0.0108	0.9903/0.9957/0.0162	-0.0057
A/MeOH (C-18)	5	0.9973/0.9980/0.0012	0.9917/0.9911/0.0019	-0.0056
OVERALL	332	0.9783/0.9890/0.0334	0.9910/0.9947/0.0119	0.0127

which the averages of r^2 values differed by 0.0366. While in some cases the Δr^2 values are negative, it should be noted that the magnitude of these differences is generally much smaller than that where positive values have been found.

Furthermore, it should also be noted that correlation coefficients found for log k' versus $E_T(30)$ regressions are likely to be reduced in magnitude because of the nature of the data being compared. Specifically, in the correlations of log k' versus $E_T(30)$ polarity, a certain amount of extra variance, not present when correlating with percent organic modifier, is introduced into the independent variable. This is due to the "external" set of data, namely the $E_T(30)$ polarity values for the solvent mixtures. Any extra variance in the x values, e.g., $E_T(30)$ polarity, will lead to a decrease in the r^2 value, even though the overall linearity of the correlation may not be different between the two methods of treatment. It is possible to estimate the relative uncertainty and hence the extra variance being introduced, by examining the standard deviations for λ_{max} determination with the diode array instrument. In separate experiments, the $E_T(30)$ polarity for 62 ternary mixtures of MeOH/ACN/H_2O were determined and thus provide an excellent set of data from which to estimate the standard deviation of λ_{max} measurements. For each mixture, a total of ten spectra were acquired, so a standard deviation can be

calculated for each set of ten. The pooled standard deviation for the entire set of 620 measurements is then given by

$$s_{pooled} = (\Sigma s_i^2 \cdot n_i)/[(\Sigma n_i) - 1] \qquad (3-3)$$

where s_i is the standard deviation of set i, and n_i is the number of λ_{max} determinations for each solvent mixture (n = 10 for all 62 sets). Using this equation the pooled standard deviation was found to be 1.16 nm. Assuming that s_{pooled} is representative of the uncertainty in λ_{max}, the uncertainty in $E_T(30)$ polarity will be given by

$$d(E_T(30)) = (-28591/\lambda_{max})^2(1.16 \text{ nm}) \qquad (3-4)$$

Thus, the total extra variance added by this uncertainty in λ_{max} will be

$$s_{extra}^2 = (n \ (dE_T(30) \cdot m)^2)/(n-2) \qquad (3-5)$$

where m is the slope of log k' versus $E_T(30)$, n is the number of log k' values in the data set, and $d(E_T(30))$ is the uncertainty in the $E_T(30)$ values. For example, where the slope was found to be 0.400 and where λ_{max} had an average value of 500 nm in six mobile phase mixtures, the maximum contribution to the total variance (s^2 of

regression) would be approximated by

$$s^2_{extra} = (6)(0.133)(0.400)^2 = 4.25 \times 10^{-03} \quad (3-6)$$

In such a case, s_{extra} would then be 0.0652. From Table 3-1, typical s values for regression of log k' versus $E_T(30)$ are in the range of 0.02 to 0.05. While the previously calculated estimate of the extra variance introduced by the $E_T(30)$ is likely to overestimate the true value, it does show the extent to which the standard error of estimate may be affected. Given this extra source of error, it is impressive that the overall median r^2 values (as shown in the bottom line of Table 3-2), which are a better way to compare two populations in which the distributions are skewed, are so close (0.9945 and 0.9946). If this extra source of error were not present, the median r^2 value for log k' versus $E_T(30)$ polarity plots would be significantly increased.

Taken by itself, this high correlation between the observed chromatographic retention and the $E_T(30)$ polarity values has little practical value. However, a wealth of information about the effect of mobile phase polarity on chromatographic retention is contained in the regression coefficients that are tabulated in Table 3-1, as well as information about the solvation of the stationary phase alkyl chains. When properly interpreted, the data

contained in Table 3-1 are of great practical value. The interpretation of these results is found in Chapter V.

Comparison with the "Carr Approach"

Sadek et al. (1985b) have recently reported correlations between retention and solvatochromic measurements of individual solutes. In terms of solute properties, molecular volume, β hydrogen bond acceptor ability, and π^* dipolarity-polarizability were found to be the best predictors of chromatographic retention, as shown below:

$$\log k' = a + b \cdot (V/100) + c \cdot \pi^* + d \cdot \beta \qquad (3-7)$$

The constants a, b, c, and d are affected by the particular column, mobile phase, and temperature. It should also be pointed out that the π^* and β parameters in equation 3-7 were adjusted with what is termed the "polarizability correction factor."

This equation is of the same form that Kamlet and coworkers have used to correlate a variety of solute/solvent properties, such as gas/liquid or octanol/water partition cofficients (Kamlet et al., 1982, 1984). However, in this particular application (Sadek et al., 1985b), solutes which might interact strongly with residual silanols present on the stationary phase (such as

amines, phenols, or anilines) were deliberately excluded from consideration. It is likely that if these solutes were included in the correlation equation, a dependence on solute α hydrogen bond donor ability would also be found (see "Suggestions for Future Research," Chapter V).

In theory, equation 3-7 could be used to predict solute retention on the basis of π^* polarity, molecular volume, and β hydrogen bond basicity. In practice, however, this is rather difficult for many compounds. Liquid chromatography is often the analytical technique of choice for compounds which are thermally labile and/or are of low volatility, rendering them unsuitable for gas chromatographic analysis. These compounds are often solids at room temperature. Thus, it is not possible to measure their π^* dipolarity/polarizability, since this is based on a solvatochromic measurement in solution. This also means that it is impossible to determine α or β values, since these are also based on solvatochromic measurements.

Therefore, this approach is severely limited by the lack of available data based on the solvatochromism of test solutes. Of course, one could evaluate π^* and β values by "back-calculating" from their retention, once the constants in equation 3-7 have been determined for solutes with known π^* and β parameters (for liquids; a comprehensive list is found in Kamlet et al., 1983).

The approach of Sadek et al. (1985b) is distinctly different from that discussed here, in that solvato-chromically measured <u>solute</u> properties are being compared with retention rather than <u>solvent mixture</u> properties. In a sense these two approaches are complementary in nature, since retention is a function of both solvent and solute properties. While not the main purpose of this research, it is worthwhile to briefly examine an alternative, multi-parameter approach to solute retention, based on the measured solvent properties (α, β, and π^* values; tabulated in Appendix D). In a sense, this approach would be the "mirror image" of that used by Sadek et al. (1985b), with the addition of the α hydrogen bond donor parameter.

To investigate the utility of this approach, a set of five retention data sets (of log k' versus percent organic modifier) were used to carry out a multiple linear regression, with log k' (at a given composition) as the dependent variable and solvent mixture α, β, and π^* values (for the same composition) as the independent variables. Since acetonitrile/water systems offer the most unusual variation in solvent properties, this system offers the best test of such an approach. The five sets were picked such that a variety of solute properties were included (protic, aprotic, nonpolar, polar). Retention data for benzene, toluene, and chlorobenzene are for a C-8 Sepralyte LC column, with acetonitrile as the mobile phase (Woodburn,

1985; also found in Appendix A), while the data for 4-nitrophenol and phenol are for a C-18 Unisil Q LC column (Hanai and Hubert, 1983; also found in Appendix A). The regression results are shown in Table 3-3, where the multiple correlation coefficients and regression coefficients are shown.

In view of the results in Table 3-3, this approach may be potentially useful. It should be noted, however, that the data sets for benzene, toluene, and chlorobenzene contained only 5 points; this is probably an insufficient quantity to make definite conclusions. The sets for 4-nitrophenol and phenol contained 7 points. It is apparent that all three coefficients vary widely with the different solutes. This is especially so for the coefficient, where phenol and nitrophenol are seen to be less retained in highly basic solvents. That is, as the basicity of the mixture increases, these solutes (which are strong hydrogen bond donors) will be more strongly solvated by the mobile phase, leading to a decrease in log k' values. With the exception of these two negative β coefficients, all other solutes are more highly retained by an increase in either the π^*, α, or β value of the mobile phase.

103

Table 3-3.
Multiple linear regression between log k' values and α, β, and π^*.

Compound	Multiple Correlation Coefficient	α	β	π^*	Constant
Benzene	0.9983	1.317	7.44	6.25	-1.08
Toluene	0.9993	0.756	8.27	7.76	-1.16
Phenol	0.9993	3.02	-1.35	1.68	-3.02
4-Nitrophenol	0.9997	4.07	-2.08	2.00	-3.73
Chlorobenzene	0.9996	2.27	6.84	7.26	-1.17

CHAPTER IV
CORRELATIONS BETWEEN CHROMATOGRAPHIC SELECTIVITY
AND MOBILE PHASE POLARITY

Experimental

For retention data other than that reported in the literature, the apparatus used is described in the experimental section of Chapter III. Methylene selectivity ($\log \alpha_{CH2}$) is defined as the change in the logarithm of the capacity factor for a given solute caused by the addition or subtraction of one methylene group (CH_2). The usual way to evaluate this is to measure the capacity factors for a homologous series (for example, alkylbenzenes, n-alcohols, or 1-nitroalkanes). One then plots the logarithm of these capacity factors with respect to carbon number of the alkyl chain, and the slope corresponds to $\log \alpha_{CH2}$. In the literature, $\log \alpha_{CH2}$ values have either already been calculated and reported or can be calculated based on the published capacity factors for homologous series. In cases where the $\log \alpha_{CH2}$ values were calculated from retention data, the program "Curve Fitter" was used, as is described in the experimental section of Chapter III.

In sharp contrast to $\log k'$ values, the variance of $\log \alpha_{CH2}$ values does not change significantly as a function of the organic modifier concentration.

Introduction

The description of methylene selectivity in RPLC has traditionally been more difficult than that of retention. In a sense, the methylene selectivity is quite similar to the retention of normal alkanes, since it is based on the measurement of the capacity factors for a homologous series. It has also been referred to as the "hydrophobic selectivity," or "nonspecific selectivity," owing to the large hydrophobicity of the methylene group. Methylene selectivity serves as a convenient measure of elution strength. Thus, knowledge of this for a given system is quite useful, since the mobile phase strength can be held constant for different organic modifiers, while the selectivity of other interactions is exploited to maximize the separation between two or more solutes.

From an energetic standpoint, the methylene selectivity ($\log \alpha_{CH2}$) is directly related to the change in the free energy of transfer caused by adding a methylene group to a molecule by the following equation:

$$\log \alpha_{CH2} = - \Delta\Delta G/2.303RT \qquad (4\text{-}1)$$

Of course, equation 4-1 applies to any form of selectivity; any two solutes that possess different free energies of transfer will be differentially retained. Methylene selectivity is thus only one aspect of

chromatographic separations. In terms of research on retention mechanisms, there are at least two distinct advantages to the study of chromatographic selectivity.

First, it can be seen that log α values are not affected by the phase ratio of the column. Different phase ratios lead to changes in capacity factors (as shown by equation 3-2). These phase ratios are a function of the bonded group chain length, the degree of surface coverage, the pore structure of the original silica, as well as the manner in which the column bed was packed. Thus, drawing conclusions about the variation in capacity factors for different columns is hindered by the number of variables to be considered. Since the methylene selectivity (or any selectivity, for that matter) is not affected by the phase ratio, any differences seen between columns are due to actual differences in the nature of the bonded phase structure.

A second advantage, obtained only in the study of methylene selectivity, is that of lack of sensitivity to the presence of residual silanols on the bonded phase surface. Residual silanols lead to anomalous retention behavior of many solutes which possess highly polar and/or hydrogen bond donor/acceptor groups. While the individual solutes that comprise the homologous series may be susceptible to these effects, the change in the capacity factor caused by additional methylene groups will still be

measurable and largely unaffected by specific interactions that each member may undergo with residual silanols.

Moreover, log α_{CH2} values are generally independent of the specific column used, such that all C-18 type bonded phases will exhibit similar behavior, implying that only the most fundamental aspects of the retention process are being probed.

Though there have been many papers published with regard to methylene selectivity, two of these are key papers and are summarized briefly in the following paragraphs. The first was published by Karger and coworkers (1976) in which the variation in log k' values for solutes such as n-alcohols were correlated with a measure of solute surface area known as the "molecular connectivity." Methylene selectivity was also discussed; a linear relationship between log α_{CH2} and percent methanol was noted, while the behavior of acetonitrile/water mixtures was found to be more complex. This behavior was ascribed to the differing natures of the two modifiers, in that methanol is a proton acceptor/donor, which leads to less disruption in the overall solvent mixture structure as the concentration is varied. The structure of acetonitrile/water mixtures is of much greater complexity, since acetonitrile does not associate with water to any extent in comparison with methanol (see Chapter II for a

discussion of this effect with the $E_T(30)$ polarity values in acetonitrile/water mixtures).

Colin et al. (1983) carried out an extensive investigation of methylene selectivity, in which a total of seven binary systems and one ternary system were explored. These measurements were then used to derive an elutropic scale of solvent strength. These new values were found to correlate quite well with Snyder's elutropic values for RPLC solvents (Snyder et al., 1979).

It is also worth noting here that homologous series have also been used in the determination of t_o (Berendsen et al., 1980). In essence, this method involves the adjustment of the t_o used to calculate the capacity factors, such that the highest correlation is obtained in a plot of log k' versus carbon number for the homologous series when the "true" t_o is reached. However, this method is subject to errors, in that the calculated t_o is in fact quite dependent upon which members of the homologous series are used to calculate t_o.

Results

In the experiments described herein, the methylene selectivity was evaluated for a C-18 bonded phase column (Ultrasphere ODS) and a styrene-divinylbenzene (polymeric; Hamilton PRP-1) reversed phase column, with methanol and acetonitrile as the organic modifiers. Also, a large body

109

of methylene selectivity data have been extracted from the literature, either directly from tabulated log α_{CH2} values, or calculated from the slope of log k' versus carbon number. The entire body of methylene selectivity discussed here appears in Appendix A. Initially, the results for the bonded phase data will be discussed; the methylene selectivity was measured by using the homologous series of benzene, toluene, ethylbenzene, and n-butylbenzene.

Ultrasphere ODS Column

In Figures 4-1 through 4-6, the results of experiments with the Ultrasphere ODS column are shown for the two organic modifiers. For methanol as an organic modifier, methylene selectivity decreases in a linear manner as the percentage or organic modifier is increased (r^2 = 0.9972 for a straight line fit of the data in Figure 4-1), while definite curvature is evident in Figure 4-2 (r^2 = 0.9897 for a linear fit). The squared correlation coefficient does decrease to 0.9884 in Figure 4-3 (versus $E_T(30)$ polarity), though this may be due in part to an increase in scatter; it is likely that the $E_T(30)$ values have contributed some extra variance (as discussed in Chapter III). The regression line drawn through the data in Figure 4-3 is given by

$$\log \alpha_{CH2} = -4.82 \pm 0.37 + 0.08817 \pm 0.0065 \ X \ E_T(30) \qquad (4-2)$$
$$(n = 5, \ s = 0.0107)$$
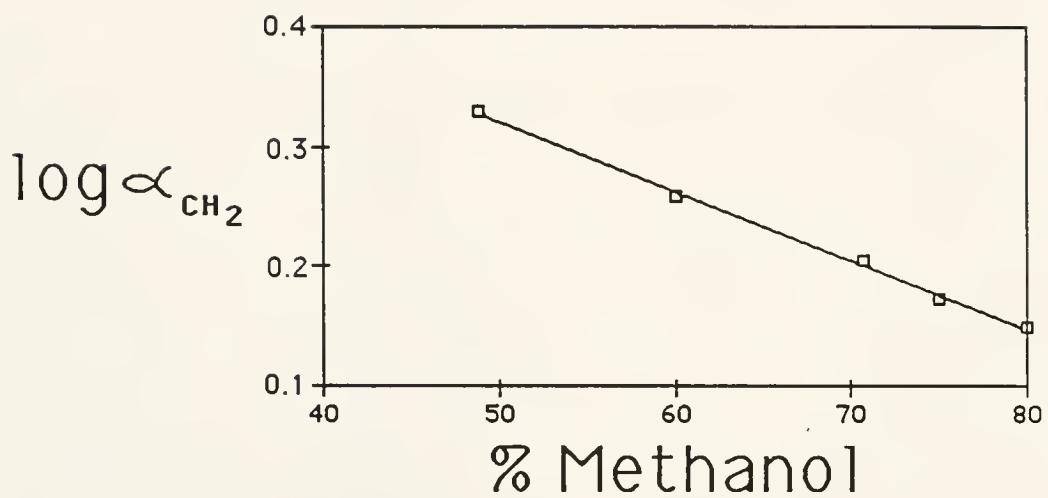
Figure 4-1. Chromatographic selectivity measurements as a
            function of percent methanol. Ultrasphere ODS
            column; flow rate 1.0 mL/min. Alkylbenzenes
            were used as the homologous series.
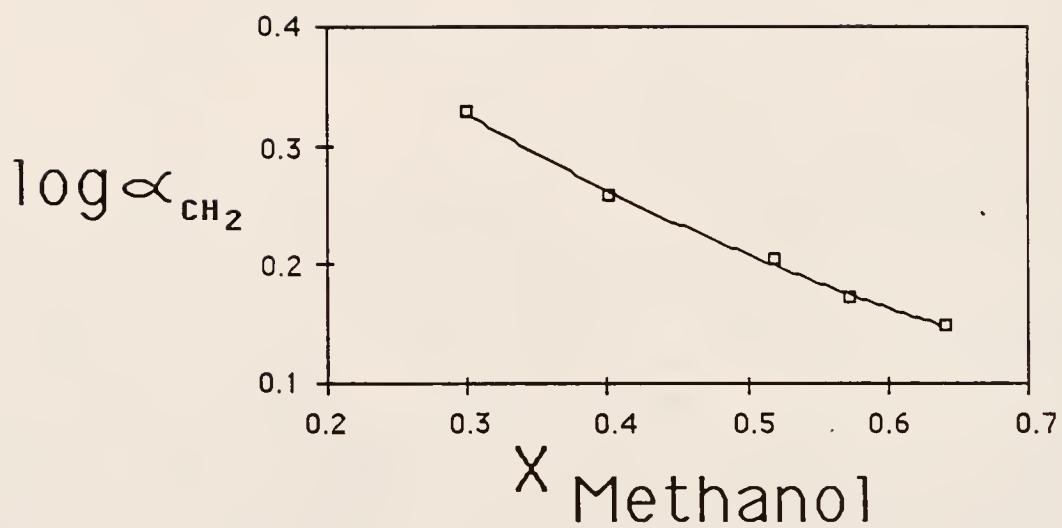
Figure 4-2.   Chromatographic selectivity measurements as a function of mole fraction of methanol. Ultrasphere ODS column; flow rate 1.0 mL/min. Alkylbenzenes were used as the homologous series.
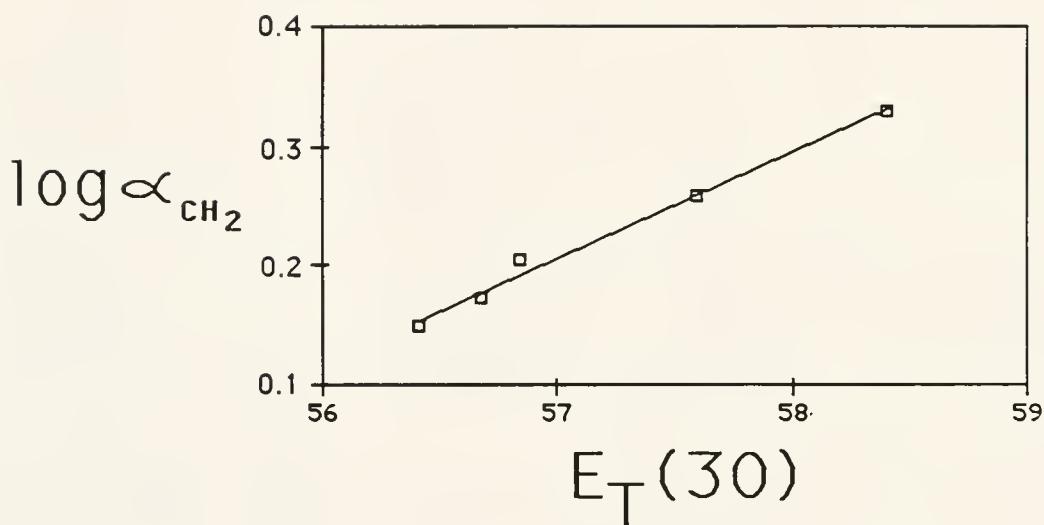
Figure 4-3. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of methanol/ water mixtures. Ultrasphere ODS column; flow rate 1.0 mL/min. Alkylbenzenes were used as the homologous series.
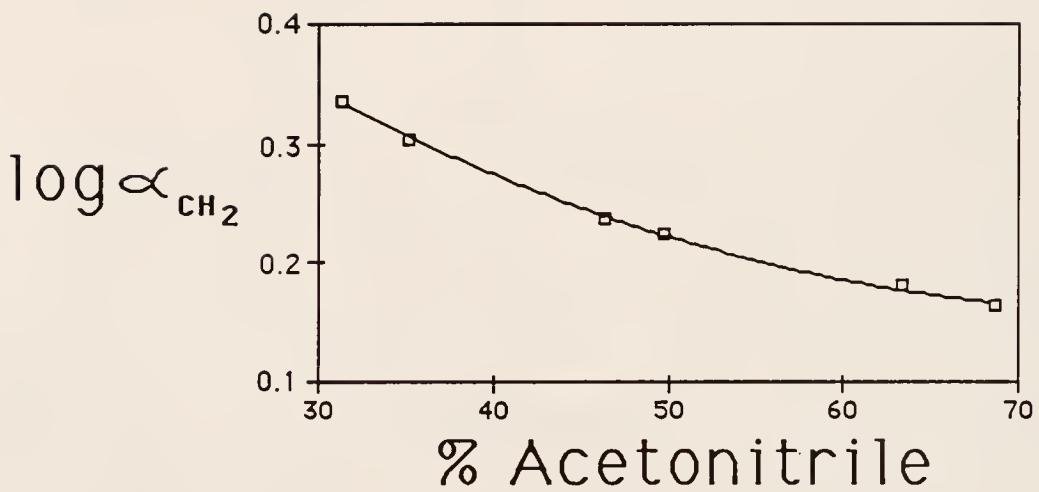
Figure 4-4.   Chromatographic selectivity measurements as a
function of percent acetonitrile. Ultrasphere
ODS   column;   flow   rate   1.0   mL/min.
Alkylbenzenes were used as the homologous
series.
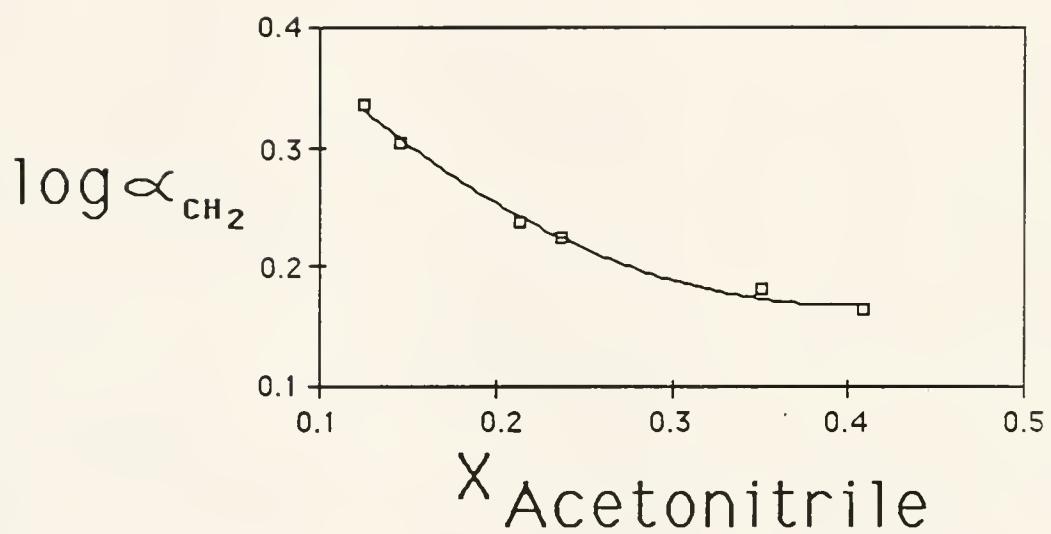
Figure 4-5.   Chromatographic selectivity measurements as a
              function of mole fraction of acetonitrile.
              Ultrasphere ODS column; flow rate 1.0
              mL/min.   Alkylbenzenes were used as the
              homologous series.
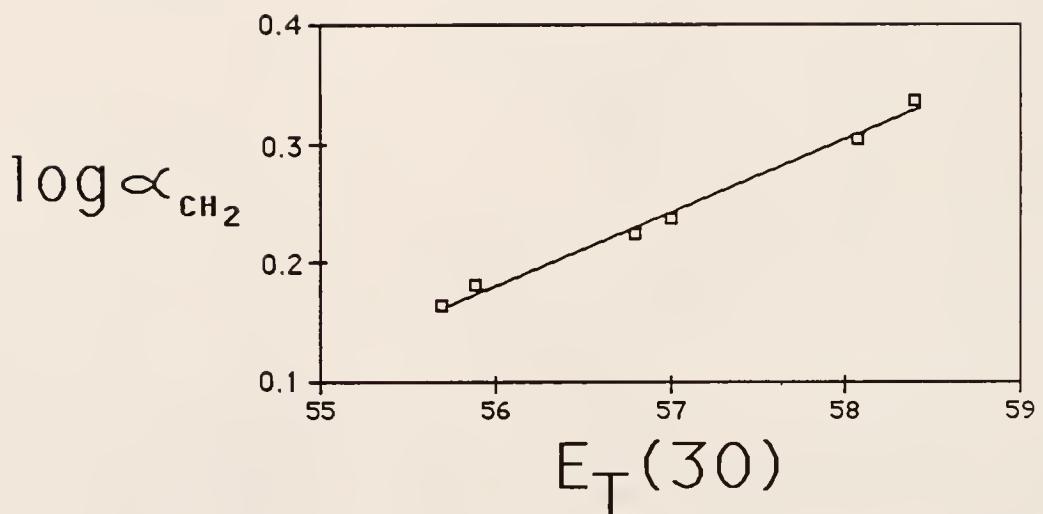
Figure 4-6. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of acetonitrile/ water mixtures. Ultrasphere ODS column; flow rate 1.0 mL/min. Alkylbenzenes were used as the homologous series.

In Figures 4-4 through 4-6, the methylene selectivity
results for acetonitrile/water mixtures are plotted with
respect to percent acetonitrile, mole fraction, and $E_T(30)$
polarity.   As   shown   in   Figure   4-4,   the   methylene
selectivity varies in a nonlinear manner; r^2 for a straight
line regression is 0.9655.   Plotting the data with respect
to mole fraction (Figure 4-5) only serves to accentuate the
curvature (r^2 = 0.9173), while plotting with respect to the
$E_T(30)$ polarity yields the best linear correlation (r^2 =
0.9919).   The regression equation for the line in Figure
4-6 is given by

$$\log \alpha_{CH2} = -3.24 \pm 0.16 + 0.06116 \pm 0.0028 \cdot E_T(30) \qquad (4\text{-}3)$$
$$(n = 6, \ s = 0.0068)$$

The ratio of the two slopes found in equations 4-2 and
4-3 is 1.44.   That is, selectivity in the methanol/water
system is more greatly affected by overall changes in
mobile phase polarity than with acetonitrile as the organic
modifier.   It should also be noted that the value of this
ratio is the same as that found for slopes of log k' versus
$E_T(30)$ (see discussion of slope ratios in Chapter V).

Literature Data

A wealth of methylene selectivity has been published
in the literature by various workers.   In some cases, the

log α_{CH2} values have been tabulated (with respect to
percent organic modifier), while in other cases the log
α_{CH2} values can be calculated from the reported capacity
factors for a homologous series. As discussed in Chapter
III, in many instances the method used to measure t_o is not
reported. Regression was carried out for data reported in
six references.

The results of the various correlations with all data
sets discussed are shown in Table 4-1. Squared correlation
coefficients are reported for each of the three comparisons
(versus percent organic modifier, versus mole fraction,
versus $E_T(30)$ polarity). This provides a way in which the
general trends of the data may be viewed. The main purpose
of these comparisons is to evaluate whether the methylene
selectivity correlates best with percentage of organic
modifier; in Figure 4-7 the results of Table 4-1 are shown
graphically. Squared correlation coefficients for log α_{CH2}
versus percent organic modifier are plotted with respect to
those found for log α_{CH2} versus $E_T(30)$ polarity. The line
drawn through Figure 4-7 corresponds to "iso-r^2" values.
That is, all points would fall along this line if all
correlation coefficients were equivalent for the two
comparisons. Thus, a point appearing above the line
denotes a better correlation when the log α_{CH2} data are
plotted with respect to percent organic modifier. Of the

Table 4-1.
Squared correlation coefficients (r^2) for log α data with respect to percent organic modifier (OM), mole fraction OM, and $E_T(30)$ polarity.

Reference/OM	n	Conc. Range	r^2 versus		
			% OM	Mole Fraction	$E_T(30)$
This work/MeOH	5	50-80	0.9972	0.9897	0.9884
This work/ACN	6	32-68	0.9655	0.9173	0.9919
Karger et al. (1978)/MeOH	13	0-100	0.9943	0.9337	0.9782
Karger et al. (1978)/ACN	8	5-80	0.9186	0.7777	0.9921
Petrovic et al. (1985)/MeOH	7	40-100	0.9945	0.9501	0.9858
Schoenmakers et al. (1981)/MeOH	5	10-100	0.9903	0.9488	0.9902
Schoenmakers et al. (1981)/ACN	7	10-80	0.9102	0.8032	0.9859
Colin et al. (1983)/MeOH	11	0-100	0.9923	0.9592	0.9486
Colin et al. (1983)/ACN	9	0-80	0.9492	0.8829	0.9776
Dufek (1984)/MeOH	10	55-100	0.9983	0.9738	0.9936
Hanai and Hubert (1985)/ACN	6	20-70	0.9855	0.9684	0.9439
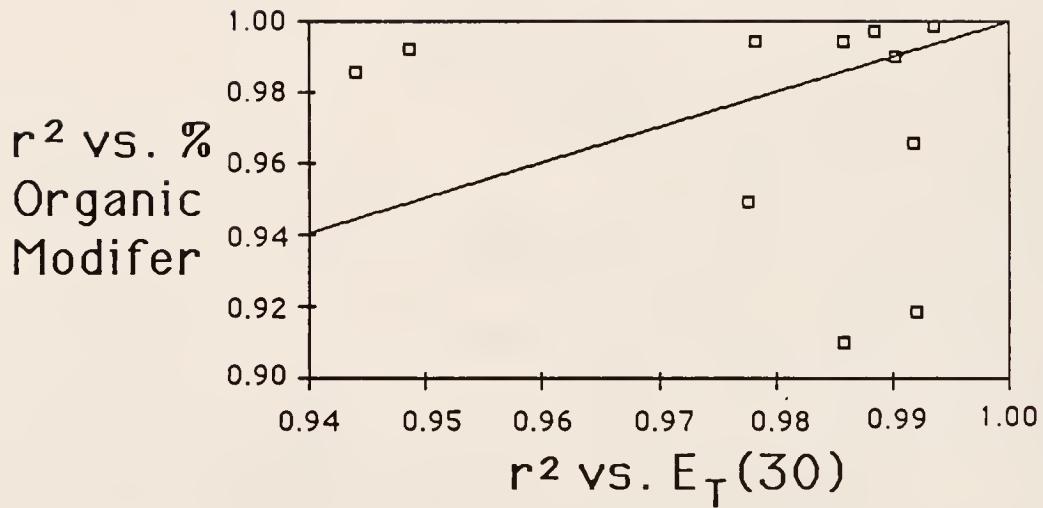
Figure 4-7. Comparison between r^2 values for plotting methylene selectivity data with respect to either percent organic modifier or $E_T(30)$ polarity.

11 data sets, in seven cases the correlation is clearly equal to or better than the "versus $E_T(30)$ polarity" comparisons.

There are a number of conclusions that can be reached regarding the data in Table 4-1. One interesting pattern that is apparent is that for every data reference where the methylene selectivity was measured with both organic modifiers, the correlation coefficients for "versus percent organic modifier" and "versus $E_T(30)$" mirror each other. That is, in methanol/water mixtures, the methylene selectivity varies most closely with the percent organic modifier, while in the acetonitrile/water system, $E_T(30)$ polarity values yield the highest correlation. In contrast to the excellent correlations seen between log k' and $E_T(30)$ polarity (Chapter III), here there is a clear-cut distinction between the two organic modifiers.

Of course, the variation with respect to percent organic modifier is also quite complex, especially with acetonitrile/water mixtures. Colin et al. (1983) found that, except for methanol/water mixtures, every system studied exhibited a nonlinear variation in log α_{CH2} with respect to percent organic modifier.

Hamilton PRP-1 Column Selectivity Measurements

While RPLC is typically done with chemically bonded silica, there are other materials that may serve as a suitable stationary phase. In recent years, a number of

polymeric reversed phase columns have become commercially available. Polymeric columns are normally prepared by the polymerization of styrene with divinylbenzene. In this manner, a highly crosslinked co-polymer is created. If the degree of crosslinking is sufficient, swelling effects will be minimized, and the column can be used under a wide variety of mobile phase conditions with minimal changes in back pressure. The polymer matrix offers a highly hydrophobi surface consisting of both aromatic ring systems and saturated alkyl chains and thus can be used in a RPLC sense.

Polymer based RPLC columns offer a number of potential advantages over their more traditional counterparts. Among these advantages is stability to a wide range of pH. Conventional bonded phases are not usable under extreme pH conditions. At pH values less than 2, hydrolysis of the siloxyl bonds leads to cleavage of the bonded chains from the silica surface, resulting in lowered retentivity and an increase in surface silanols. At a pH of greater than 8, the solubility of the silica in the mobile phase increases dramatically, ultimately leading to a degredation of the packing structure of the column and resultant lowered efficiencies and retentivity. Unlike silica based columns, polymer based columns are stable to pH levels of 1-13 or high concentrations of buffer salts, with no degredation in performance.

Another aspect of these columns that can be of use is their preferential retention of the aromatic compounds. Apparently the presence of aromatic π-electron orbitals leads to preferential retention of aromatic solutes, leading to different chromatographic selectivity than that found with conventional RPLC columns.

A final advantage is the lack of any silanol groups whatsoever. As mentioned in Chapter I, the presence of these groups on the surface of conventional bonded phases can be a problem with highly polar solutes, which will in effect be retained by a dual adsorption/partitioning mechanism. Thus, polymeric columns are, by virtue of their composition, entirely free of these troublesome residual silanols.

Given the above factors, one might logially inquire as to whether polymeric columns are likely to replace the (currently) more popular bonded phases. This is not likely in the immediate future, owing to their generally lower efficiencies. Though small particle sizes (5 and 10 micron mean diameter) are now available, the number of plates delivered per unit length is still significantly less than conventional columns, and thus in situations where large plate numbers must be generated, their utility is diminished. This lowered efficiency is most likely due to an increase in the resistance to mass transfer, since the entire volume of the particles comprises the stationary

phase, and thus diffusion of the solute into the interior of the particles is much slower than the corresponding open pores found with silica based matrices.

The experiments carried out with a Hamilton PRP-1 column are discussed in the following text and involved measuring the methylene selectivity over 0-100% methanol and 0-80% acetonitrile. Owing to the high retentivity of the polymer matrix with respect to aromatic compounds, it was found that the homologous series of alkylbenzenes is unsuitable at low concentrations of organic modifier (i.e., less than 60% v/v). Therefore, nitroalkanes (nitromethane through nitrohexane) were used to measure log α_{CH2} values, and were found to be usable over the entire range of organic modifier concentration.

In light of the fact that the polymeric column has a preferential retentivity toward aromatic compounds, it was necessary to insure that the use of a different homologous series would not significantly affect the measurement of log α_{CH2}. Both nitroalkanes and alkylbenzenes were used to measure this at three different concentrations in the two organic modifiers. Concentrations were chosen such that the alkylbenzenes could still be used to measure the methylene selectivity (>60% organic modifier). The results of these comparisons are found in Table 4-2 and are plotted in Figure 4-8. The slope of the line drawn through the
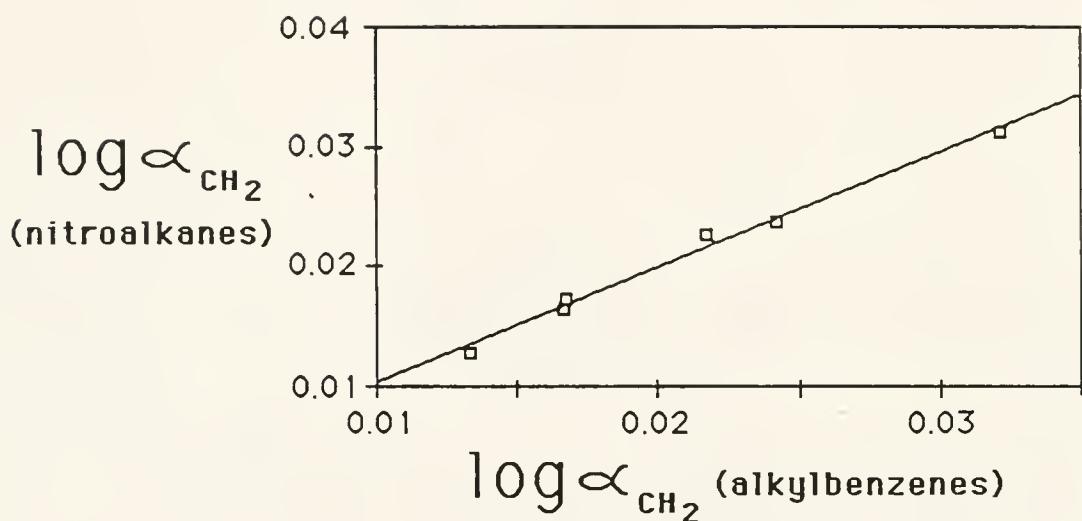
Figure 4-8.   Comparison   between   methylene   selectivity
              results obtained with either 1-nitroalkanes or
              alkylbenzenes   as   the   homologous   series.
              Hamilton PRP-1 (polymeric) column; flow rate
              1.0 mL/min.

data was 1.02±0.17 with a y-intercept of -0.005±0.04. Based on these results, it does not appear that the measurement of methylene selectivity is significantly biased by the homologous series used to measure it and is consistent with results published for conventional bonded phases.

Table 4-2.
Comparison of log α values as measured by nitroalkanes and alkylbenzenes for a Hamilton PRP-1 column.

Mobile Phase	log α_{CH2} Alkylbenzenes	Nitroalkanes
50% ACN	0.22	0.23
65% ACN	0.17	0.16
80% ACN	0.13	0.13
70% MeOH	0.32	0.31
80% MeOH	0.24	0.24
90% MeOH	0.17	0.17

One distinct disadvantage of the use of nitroalkanes is their generally low absorption of light in the UV region. For example, the molar absorptivity of nitromethane is only 18.6 in ethanol, with a λ_{max} of 271 nm. Nevertheless, it was found that by increasing the concentration of nitroalkanes in the injected standards to approximately 5 mg/mL (20 microliter sample volume; 100 μg injected), a 254 nm UV detector could still be used to detect the nitroalkanes. The peak shapes did not appear to

be distorted by the large amount of injected solute; this may be a reflection of the nature of the polymeric stationary phase, since a higher loading level should (in theory) be tolerated.

A representative example of the measurement of the methylene selectivity is shown in Figure 4-9, where data for 80% methanol have been plotted. "Carbon #" denotes the size of the nitroalkane (1 = nitromethane, 2 = nitroethane, etc.). Correlation coefficients for the three comparisons discussed herein appear in Table 4-3.

Table 4-3.
Correlations between log α and percent organic modifier (OM), mole fraction organic modifier (MF OM), or $E_T(30)$ polarity for a Hamilton PRP-1 polymeric column.

Mobile Phase	n	r^2 versus % OM	r^2 versus MF OM	r^2 versus $E_T(30)$
Methanol	11	0.9989	0.9498	0.9692
Acetonitrile	9	0.8816	0.7352	0.9731

The results of the measurements of log α_{CH2} for methanol/water mixtures appear in Figures 4-10 through 4-12. Figure 4-10 shows that the methylene selectivity decreases in a highly linear fashion as the percent (v/v) of methanol is increased. The regression line corresponds to

$$\log \alpha = -5.24 \pm 0.34 + 0.0970 \pm 0.0058 \cdot E_T(30) \quad (4\text{-}4)$$

$$(n = 11, \; s = 0.0468)$$

Plotting $\log \alpha$ with respect to mole fraction leads to strong curvature. Also, as shown in Figure 4-12, plotting with respect to the $E_T(30)$ also results in curvature, though at higher $E_T(30)$ polarities (lower methanol concentrations) the behavior is nearly linear.

The results for the same measurements with acetonitrile as an organic modifier appear in Figures 4-13 to 4-15. Here the curve shapes are distinctly different than those seen with methanol. Instead of linearity versus percent organic modifier, strong curvature is seen; this behavior in the two organic modifiers is quite similar to that seen with standard bonded phase columns. The change in $\log \alpha_{CH2}$ is most pronounced at low concentrations of organic modifier. Finally, in Figure 4-15, the selectivity increases in a nearly linear manner, with strong curvature seen at high $E_T(30)$ polarity values (low acetonitrile concentration). If the data in Figures 4-15 are fitted to a straight line model, the resultant regression line is given by

$$\log \alpha = -4.58 \pm 0.31 + 0.0847 \pm 0.0053 \cdot E_T(30) \quad (4\text{-}5)$$

$$(n = 9, \; s = 0.0411)$$

128
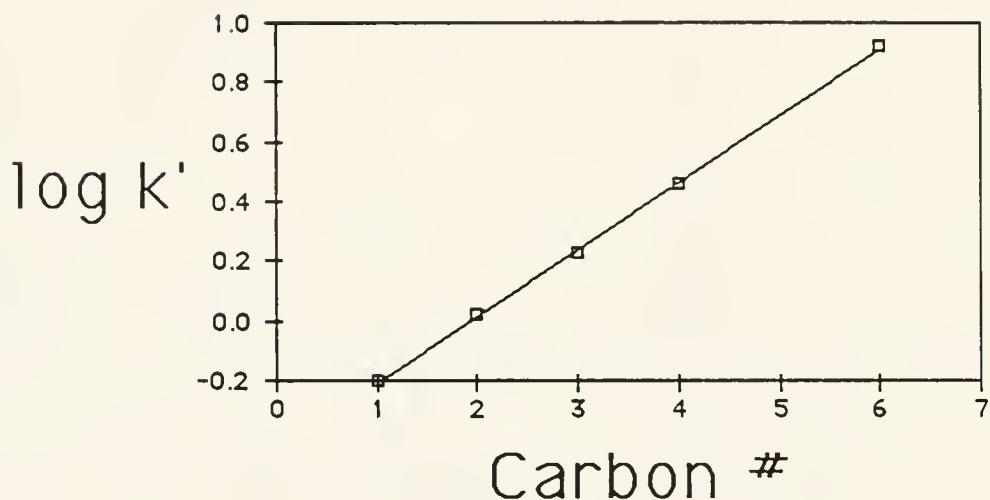
Figure 4-9. Example of the measurement of methylene selectivity with nitroalkanes as the homologous series. Hamilton PRP-1 column; flow rate 1.0 mL/min; 80% methanol as the mobile phase.
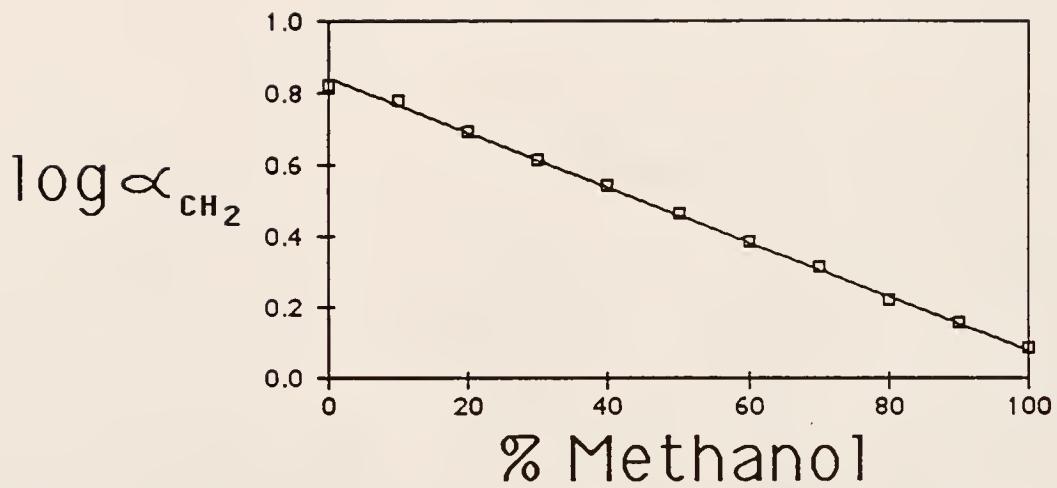
Figure 4-10. Chromatographic selectivity measurements as a function of percent methanol. Hamilton PRP-1 column; flow rate 1.0 mL/min. Nitroalkanes were used as the homologous series.
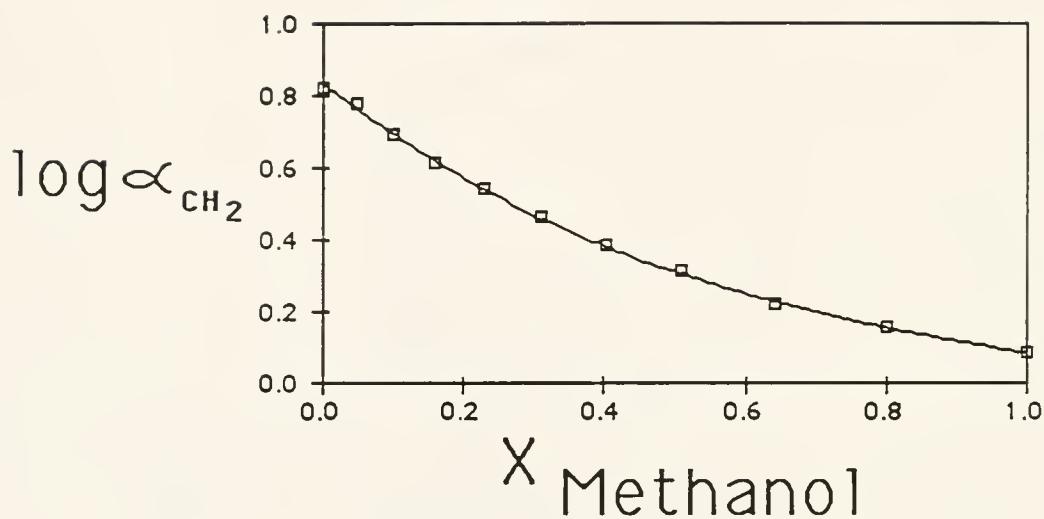
Figure 4-11. Chromatographic selectivity measurements as a
function of mole fraction of methanol.
Hamilton PRP-1 column; flow rate 1.0
mL/min.   Nitroalkanes were used as the
homologous series.
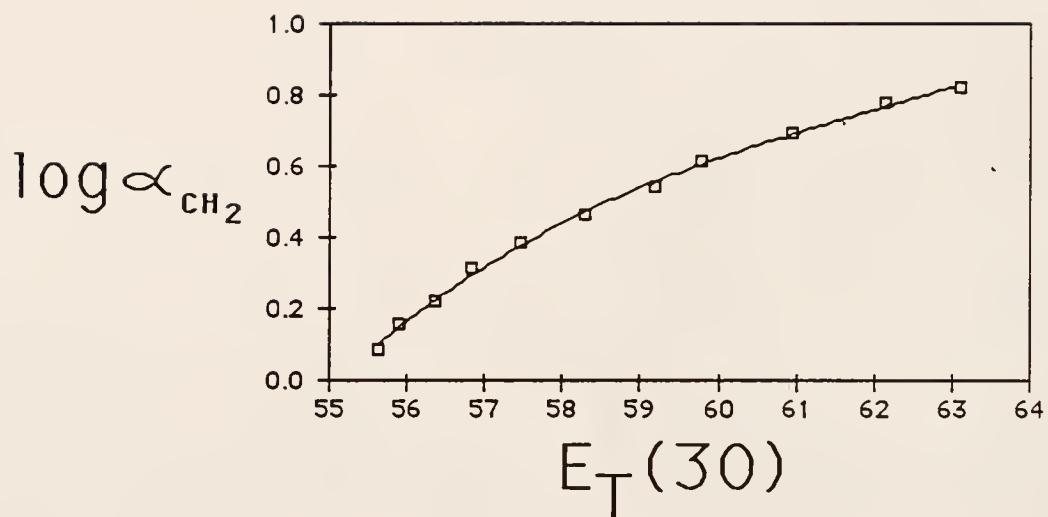
Figure 4-12. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of methanol/water mixtures. Hamilton PRP-1 column; flow rate 1.0 mL/min. Nitroalkanes were used as the homologous series.
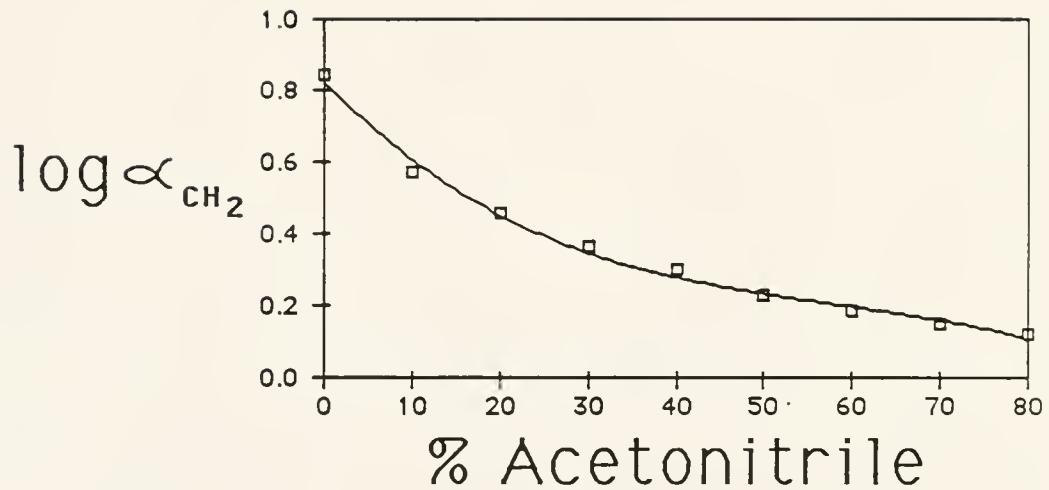
Figure 4-13. Chromatographic selectivity measurements as a function of percent acetonitrile. Hamilton PRP-1 column; flow rate 1.0 mL/min. Nitroalkanes were used as the homologous series.
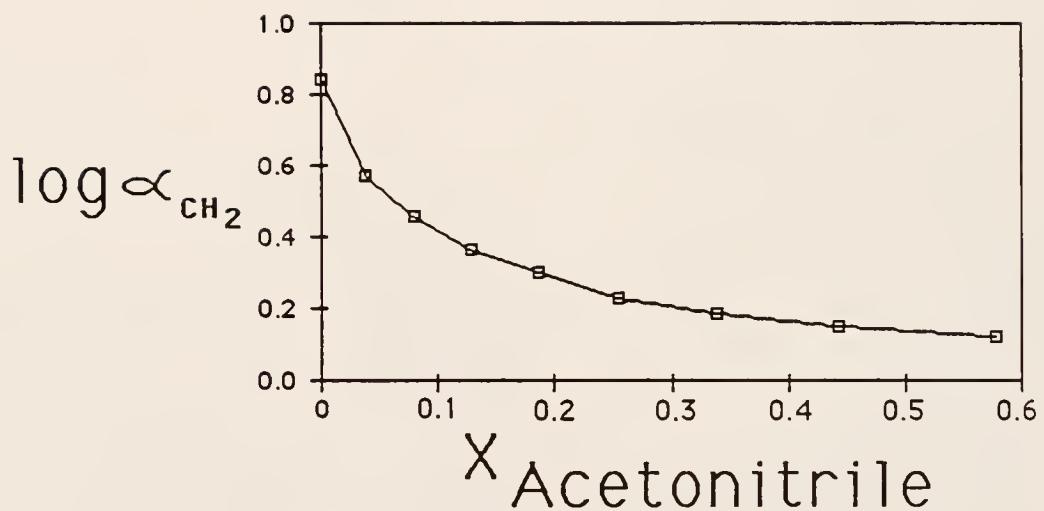
Figure 4-14.   Chromatographic selectivity measurements as a
              function of mole fraction of acetonitrile.
              Hamilton   PRP-1   column;   flow   rate   1.0
              mL/min.   Nitroalkanes   were   used   as   the
              homologous series.
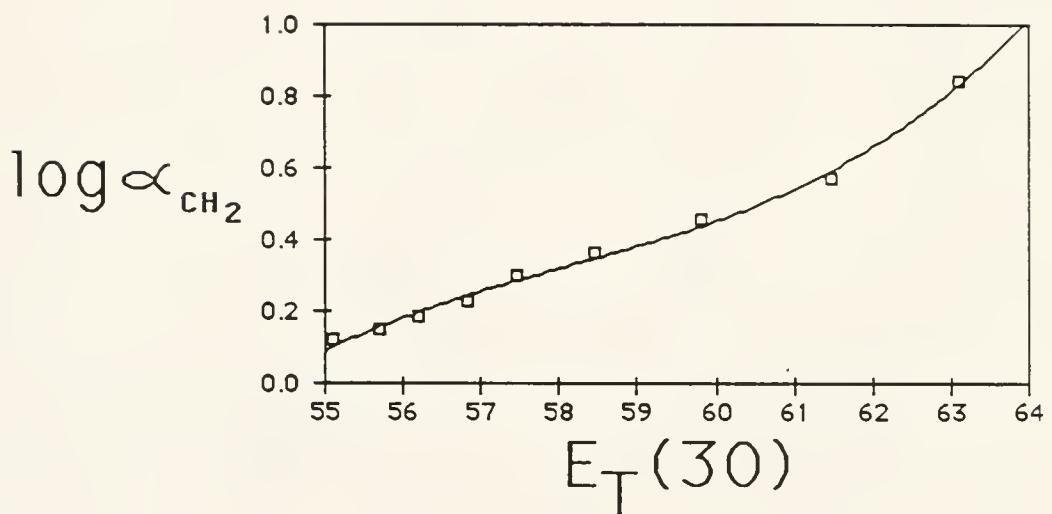
Figure 4-15. Chromatographic selectivity measurements as a
function of $E_T(30)$ polarity of acetonitrile/
water mixtures. Hamilton PRP-1 column; flow
rate 1.0 mL/min. Nitroalkanes were used as
the homologous series.

At 100% organic modifier concentration, the log α_{CH2} values are 0.0850 and 0.0754 for methanol and acetonitrile, respectively. This is as one might expect, owing to the greater "strength" of acetonitrile as an organic modifier in RPLC. Lastly, it should be noted that log α_{CH2} values for this column are significantly higher at a given percent organic modifier than that of the Ultrasphere ODS bonded phase column. This shows that the polymeric surface is even more "hydrophobic" than the bonded phases, since the free energy of transfer of a methylene group is larger in magnitude.

CHAPTER V
DISCUSSION AND CONCLUSIONS

The purpose of the research described here is to examine the exact role that the polarity of the mobile phase plays in the retention of solutes in reversed phase liquid chromatography. Study of chromatographic retention mechanisms can involve examination of the stationary phase (e.g., FTIR and NMR experiments), the mobile phase (as discussed herein), or some combination of the two (i.e., retention measurements). In general, the $E_T(30)$ polarity serves as a good measure of the strength of the binary aqueous/organic mobile phases used in RPLC. Specifically, plots of log k' versus $E_T(30)$ polarity are better descriptors of chromatographic retention than commonly used plots of log k' versus percent organic modifier.

Plotting log k' versus $E_T(30)$ yields two coefficients (slope and y-intercept); the slope has the most physical significance, in that it measures the change in free energy of transfer of the solute as a function of changes in $E_T(30)$ polarity. Y-intercepts signify the log k' value where the $E_T(30)$ polarity is zero. Of course, an $E_T(30)$ polarity of zero is meaningless; this would correspond to

equivalence of energy of the ground and excited states of the ET-30 molecule. A more useful intercept would be calculable if the UV/VIS absorption spectrum of ET-30 could be measured in the absence of any solvation (i.e., in the gas phase). If this were known, then the log k' value for a completely inert mobile phase could be established, providing information about the stationary phase. Unfortunately, the $E_T(30)$ molecule is quite nonvolatile, with an indefinite melting point in the region of 273°C (Dimroth et al., 1963a). Gas-phase π* values have been reported (Abboud et al., 1984; Essfar et al., 1982); this was possible since the solutes that define the π* scale are much more volatile (e.g., 4-ethylnitrobenzene, which is a liquid at room temperature). Despite the lack of a gas-phase $E_T(30)$ polarity value, there is still much information provided by the slope of log k' versus $E_T(30)$ polarity.

With regard to these slope values, if the change in the free energy of the retention process were exactly the same as the change in the $E_T(30)$ polarity, the slope of the resultant line would be 0.73 at 25°C (it would be exactly 1.0 if instead of log k', 2.303 RT·log k' were being plotted).

The slope and y-intercept values shown in Table 3-1 for the 332 sets of chromatographic retention data examined here provide much information about the effect of changing

mobile phase polarity on retention. There are systematic trends in the slope based on the size or type of molecule being examined. Also, information about the stationary phase solvation is also provided indirectly, as will be discussed later in this chapter. Although all slope and y-intercept values have been tabulated in Table 3-1, it is instructive to discuss small subsets of this master table, in order to highlight the effects of increasing solute size and nature of the column upon sensitivity to changes in mobile phase polarity.

As the size of the molecule is increased, the magnitude of the slope and y-intercept also increase. This effect is demonstrated in Table 5-1, in which the regression results for alkylbenzenes in the two solvent systems have been tabulated. In Table 5-1, "Data set #" refers to the line number of Table 3-1 from which the data are taken. This increase in sensitivity to changes in $E_T(30)$ polarity is a linear function of the size of the alkyl chain, as demonstrated in Figures 5-1 and 5-2, where the slopes for the two systems are plotted with respect to carbon number. The regression equations for the two lines are

(in MeOH) slope = 0.383±0.002

$$+ 0.0884\pm0.0008 \cdot \text{Carbon \#} \qquad (5\text{-}1)$$

(n = 4, s = 0.00074)

Table 5-1.
Effect of increasing solute size upon sensitivity to changes in $E_T(30)$ polarity for alkylbenzenes.

Data Set #/Solute	Column/Mobile Phase	Slope (X 10^{-2})	-(y-int)
5/Benzene	A/ACN (C-18)	34.5	19.0
12/Toluene	"	40.9	22.5
7/Ethylbenzene	"	47.2	25.8
8/Isopropylbenzene	"	52.3	28.5
6/n-Butylbenzene	"	60.2	32.7
328/Benzene	B/MeOH (C-18)	38.2	21.4
331/Toluene	"	47.4	26.5
332/Ethylbenzene	"	55.8	31.0
330/Isopropylbenzene	"	63.0	35.0
329/n-Butylbenzene	"	73.7	40.8

(in ACN) slope = 0.345±0.001

$$+ \ 0.0642±0.0002 \cdot \text{Carbon} \ \# \qquad (5\text{-}2)$$

(n = 4, s = 0.00087)

An interesting effect shown by equations 5-1 and 5-2 is that the slope in methanol is 1.37 times that found for acetonitrile. This finding suggests different solvation of the stationary phase in the two systems (see discussion in the following section of this chapter). In a sense, this type of plot is a correlary to the measurement of methylene selectivity, where log k' is plotted versus carbon number at a single organic modifier concentration (Chapter IV; Figure 4-9). However, in this instance the organic modifier concentration is being changed, so a plot of the slope versus carbon number corresponds to something different. This is perhaps best termed the "methylene polarity selectivity." That is, what is being measured is the effect of the overall mobile phase polarity on the methylene group selectivity.

Another illustration of the effect of increasing solute size is shown in Table 5-2, where the regression coefficients for halobenzenes in the two solvent systems are tabulated. As with the alkylbenzenes, the magnitude of the slope and y-intercept increase in a regular fashion as the halogen atom attached to benzene increases in size. This general effect seen for all solutes is consistent with

Table 5-2.
Effect of increasing solute size upon sensitivity to changes in $E_T(30)$ polarity for halobenzenes.

Data Set #/Solute	Column/Mobile Phase	Slope (X 10^{-2})	-(y-int)
179/Fluorobenzene	D/ACN (C-8)	36.2	20.2
177/Chlorobenzene	"	40.7	22.6
176/Bromobenzene	"	42.0	23.2
180/Iodobenzene	"	44.5	24.6
291/Fluorobenzene	D/MeOH	50.8	29.0
289/Chlorobenzene	"	57.1	32.4
288/Bromobenzene	"	59.1	33.5
292/Iodobenzene	"	62.5	35.3
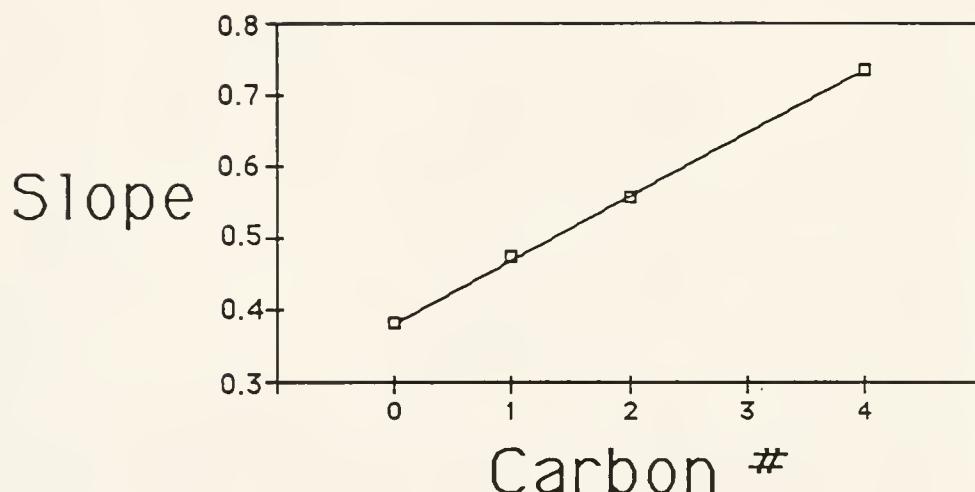
Figure 5-1. Slope of log k' versus $E_T(30)$ polarity as a function of carbon number for methanol/water mixtures. Ultrasphere ODS column; alkylbenzenes.
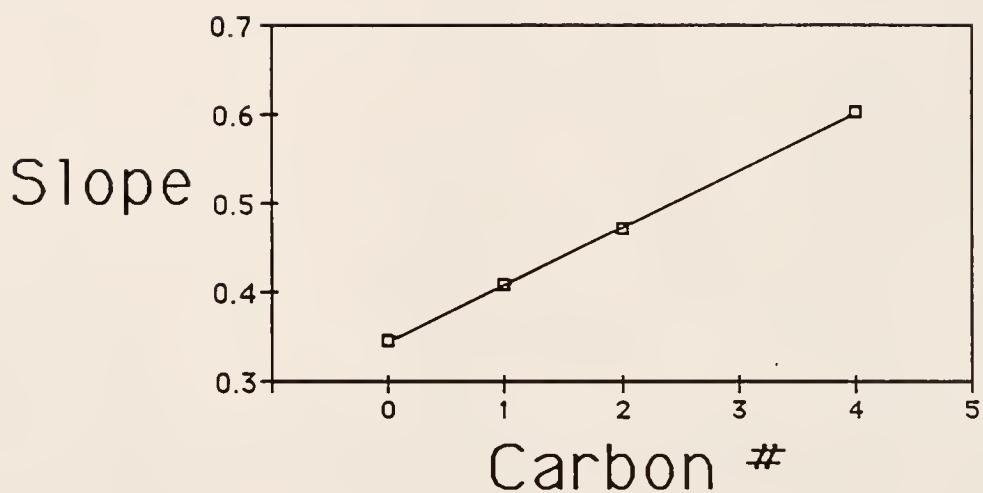
Figure 5-2. Slope of log k' versus $E_T(30)$ polarity as a function of carbon number for acetonitrile/ water mixtures. Ultrasphere ODS column; alkylbenzenes.

the solvophobic theory of Horvath, in which increasing size
(referred to as "hydrocarbonaceous surface area") would be
expected to increase retention (Horvath et al., 1976).

Another aspect to be examined here is the dependence
on the sensitivity on the nature of the column. Again, as
with the molecular size, the length of the carbon chain of
the bonded phase appears to also increase the sensitivity
of the retention process to changes in the $E_T(30)$
polarity. This is shown in Table 5-3 where the slope and
y-intercept values for phenanthrene for various columns
(C-2 through C-18) have been tabulated. In Table 5-4, the
same information is shown for ethylbenzene. The magnitude
of the slope appears to increase at the highest rate when
going from C-2 through C-8 type bonded phases, while the
increase is much less in going from C-8 to C-18. This
suggests that beyond a certain length (between 4 and 8
carbon atoms) of the alkyl chain, a steady state of
solvation is achieved, so that only the outer atoms in the
chain are solvated by the mobile phase. This is consistent
with the work of Berendsen and De Galan (1980), who found
that there is a "critical carbon number" for the chain
length beyond which retention is not greatly affected. It
was found to be slightly dependent on the solute size, with
an average of approximately eight. Croo et al. (1985)
reported similar results for some pharmaceutical compounds.

Table 5-3.
Comparison of slope and y-intercept values for
log k' versus $E_T(30)$ polarity for phenanthrene.

Column/Solvent	Slope (X 10^{-2})	-(y-int)
A/ACN (C-18)	60.0	32.9
B/ACN (C-2)	48.9	27.2
C/ACN (C-4)	50.2	27.8
D/ACN (C-8)	58.6	32.2
E/ACN (C-18)	57.6	31.4
B/MeOH (C-2)	69.2	39.6
C/MeOH (C-4)	73.9	41.8
D/MeOH (C-8)	80.7	45.4

Table 5-4.
Comparison of slope and y-intercept values for
log k' versus $E_T(30)$ polarity for ethylbenzene.

Column/Solvent	Slope (X 10^{-2})	-(y-int)
A/ACN (C-18)	47.2	25.8
B/ACN (C-2)	34.4	19.1
C/ACN (C-4)	37.8	20.9
D/ACN (C-8)	45.6	25.2
E/ACN (C-18)	48.9	26.8
B/MeOH (C-2)	46.1	26.5
C/MeOH (C-4)	51.8	31.1
D/MeOH (C-8)	64.0	36.2

Finally, aromatic substitutional isomers appear to
have nearly identical sensitivity to changes in the
polarity of the mobile phase. This is illustrated quite
clearly with the isomers of ethylphenol (sets 27, 35, and
40), in which the 2-, 3-, and 4-ethylphenols have slope and
y-intercepts of 0.342/0.339/0.341 and -18.8/-18.6/-18.7,
respectively.

Chromatographic retention is, from a thermodynamic standpoint, the result of differences in free energy of the solute as it transfers between the mobile and stationary phases. This can be expressed quantitatively by the following equation:

$$\log k' = -2.303 \ \Delta G/RT + \log (\phi) \qquad (5\text{-}3)$$

where ϕ is the phase ratio of the column (V_s/V_m). Of course, the ΔG term can be separated into the individual contributions of ΔH and ΔS, resulting in the following equation:

$$\log k' = -2.303 \ \Delta H/RT + 2.303 \ \Delta S/R + \log (\phi) \quad (5\text{-}4)$$

Thus, plotting $\log k'$ versus $1/T$ yields the enthalpic change for the retention process. These plots can also be used to assess whether the retention process occurs by a dual mechanism, which is indicated by nonlinearity.

It can be seen in equation 5-4 that the ΔH is unaffected by the phase ratio of the column. Woodburn (1985) has reported the enthalpies of transfer for a wide variety of solutes and columns as a function of mobile phase composition. Linear regression was done for these values versus the $E_T(30)$ values for the same solvent mixtures. These results are summarized in Table 5-5, where

the r^2 values for plots of ΔH versus $E_T(30)$ polarity have been tabulated.

Table 5-5.
Correlations between enthalpy of transfer (ΔH) and $E_T(30)$ polarity values. Enthalpic data from Woodburn (1985).

Column Type/Mobile Phase	n	Mean r^2 Value
C-2/ACN	21	0.9608
C-4/ACN	22	0.9848
C-8/ACN	22	0.9538
C-2/MeOH	21	0.8406
C-4/MeOH	21	0.9706
C-8/MeOH	22	0.9439

In general, the correlation coefficients are much lower than the corresponding plots of log k' versus $E_T(30)$ polarity (Table 3-2). Sander and Field (1980) have studied the variation in enthalpic and entropic contributions to retention as a function of mobile phase composition for a C-18 µBondapack column. For isopropylbenzene, the enthalpy of transfer was found to increase in a nearly linear fashion as the concentration of methanol was increased from 45 to 100%, while N,N-diethylaniline exhibited less predictable behavior.

There are only a handful of reports in the literature regarding the correlation between chromatographic and spectroscopic properties of solutes. One example is the work of Fetzer and Biggs (1985), in which the spectral

properties of peropyrene polycyclic aromatic hydrocarbons (PAHs) were correlated with anomalous retention behavior. Anomalous retention behavior in this instance is defined as not being consistent with molecular size. The retention of PAHs can usually be predicted on the basis of molecular size. It was found that the electronic spectra of PAHs in different solvent mixtures were not significantly changed, except in cases where anomalous retention behavior (such as early elution with respect to similar-sized PAHs) was observed. For these cases, there was evidence that changes in the planarity of the ring system were induced by the solvent. These changes in planarity then led to decreased retention as well as a change in the spectral peak shapes.

Wirth et al. (1983) reported correlations between the bandwidth of electronic 0-0 bands of the first excited singlet states of dimethylnapthalenes and their relative retention. As a measure of the repulsive/attractive forces between the mobile phase and the test solute, the bandwidth for each solute was measured in two different mobile phases (n-dodecane and 85% acetonitrile/water). The difference in bandwidths in the two systems was then compared to retention times of the various solutes, and a linear relationship was found. Solutes with the greatest bandwidth difference were found to be the most highly retained.

These two papers clearly demonstrate that there is a relationship between spectroscopically observable solvation of a test probe and its chromatographic retention. It follows then that there should also be a relationship between spectroscopically observable mobile phase properties and chromatographic retention. With regard to the $E_T(30)$ polarity scale, one is observing (primarily) changes in the solvation of the ground electronic state of the molecule caused by changes in the solvent environment. According to the Franck-Condon principle, the time scale of optical absorption is such that the solvent molecules do not have sufficient time to re-orient themselves around the excited molecule. Thus, since the ground state orbital electrons of $E_T(30)$ are much more unequally distributed (shown by a dipole moment of approximately 15 Debye) than that of the excited state (2 Debye), the solvation of the ground state is more sensitive to the surrounding solvent (Reichardt et al., 1980). Qualitatively, this is probably the most accurate way in which to measure the mobile phase polarity (as opposed to bulk properties such as dielectric constant, viscosity, etc.), since chromatographic retention is directly related to the free energy of solvation (ground state property) of the solute in the two different phases.

Stationary Phase Effects

As mentioned previously, the slopes of log k' versus $E_T(30)$ polarity are different for the same solute and column in the two mobile phase systems. For the retention data sets examined here, there were 89 cases in which data for a given solute was available in both methanol/water and acetonitrile/water mixtures. Thus, a slope ratio can be calculated for each of these 89 cases. These slopes for the two organic modifiers vary in a sytematic manner, in that the ratio of the slopes found in the methanol/water mixtures are consistently greater than those found with acetonitrile/water mixtures. In Table 5-6 the average ratios of the slope for a given solute and column are shown. Overall, the average ratio was found to be 1.43 (s = 0.06).

Table 5-6.
Ratio of slopes for a given solute and column with methanol and acetonitrile as organic modifiers.

Column (type)	n	Average Ratio Slope (MeOH)/Slope (ACN)	s
A (C-18)	5	1.18	0.045
F (C-8)	25	1.49	0.083
B (C-2)	19	1.41	0.051
C (C-4)	20	1.49	0.041
D (C-8)	20	1.40	0.028

Initially, the higher slope found with methanol/water mixtures would appear to be at odds with the general notion

of acetonitrile being a "stronger" solvent for reversed phase liquid chromatography. However, this observation is in fact entirely reasonable, if one considers the effect of solvation of the stationary phase. That the slope is different for the two organic modifiers is evidence of the importance of the stationary phase in the retention process. If the stationary phase was truly "inert," the slopes should be equal, as the $E_T(30)$ measures the actual solvating power of the mobile phase, and iso-$E_T(30)$ values should have equivalent elution strengths. An example of ET-30 measuring a solution property was published by Elias et al. (1981), in which the $E_T(30)$ polarity values were correlated with a reaction rate constant. Also, heats of solution at infinite dilution have been correlated with the $E_T(30)$ polarity of some pure solutes (as discussed in Chapter I). Here, actual retention is a result of the free energy change as a solute transfers from the mobile to stationary phases. Thus different slopes of log k' versus $E_T(30)$ must indicate that the solute is experiencing a different environment in the stationary phase as the organic modifier is changed from methanol to acetonitrile. The greater slope in methanol water shows that for an equal change in mobile phase polarity, retention is affected to a greater extent in methanol/water systems.

This is entirely consistent with results of earlier workers who have measured the distribution isotherms for organic modifiers used in reverse phase chromatography. In particular, McCormick and Karger (1980a, 1980b) showed that at all concentrations of organic modifier, the stationary phase contains a higher concentration of acetonitrile.

Thus, the higher slope (versus polarity) found in methanol/water systems is a reflection of the fact that there is much less methanol in the stationary phase, so a change in the mobile phase polarity will influence chromatographic retention to a much greater degree than in acetonitrile/water systems, where a change in overall mobile phase polarity is compensated by more or less solvation of the stationary phase. Herein lies one of the advantages of plotting retention versus $E_T(30)$ polarity, in that the effect of the stationary phase can be deconvoluted from that of the mobile phase. Chromatographic retention is a function of the nature of both the stationary and mobile phases, while the polarity measurements are a function of the mobile phase only.

Another way in which the regression coefficients for log k' versus $E_T(30)$ polarity can be applied is by calculating intersection points of the lines defined by log k' versus percent organic modifier. That is, the intersection points for the lines described for various

solutes can provide information about the polarity of the stationary phase.

There are two distinct groups of intersection points that can be described for each column. The first, involving the lines described for one solute and column in the two mobile phase systems, provides very little information. That is, the two lines will intersect at the point where the log k' values are equivalent, which will occur only when the mobile phase is pure water, i.e., $E_T(30) = 63.1$ kcal/mole.

The second type of intersection point is that for different solutes with the same organic modifier and column. In this instance, the intersection point corresponds to the $E_T(30)$ solvent polarity where the log k' values are equal. In other words, it is at this polarity where the solutes "see" the same difference between the stationary and mobile phases, and hence the $E_T(30)$ polarity of this point provides a measure of the polarity of the stationary phase. Of course, for each set of n lines, there will be a total number of intersection points given by

$$N_{total} = (n-1) + (n-2) + (n-3) + \ldots 1 \qquad (5-5)$$

A problem arises when comparing lines of very similar slope, since the intersection point for lines that are

nearly parallel will be extremely susceptible to minor fluctuations in the slope values. One way in which to address this problem is to use lines which differ significantly in their slopes, and thus will provide better estimates of the true point of intersection. Thus, homologous series lend themselves to this type of calculation, since the slopes change in a regular fashion with the size of the alkyl chain. Moreover, use of only one family of compounds reduces the effect of any specific interactions with the stationary phase.

The results of calculations of the intersection points for the homologous series of alkylbenzenes are shown in Table 5-7. Confidence limits reported are for the 95% level. A general trend can be seen in that the $E_T(30)$

Table 5-7.
Intersection points for log k' versus $E_T(30)$ for alkylbenzenes.

Column/Mobile Phase	$E_T(30)$*	n
A (C-18)/ACN	53.3±0.8	6
" /MeOH	54.5±0.6	6
B (C-2)/ACN	54.8±0.3	10
" /MeOH	55.3±0.1	10
C (C-4)/ACN	54.2±0.5	15
" /MeOH	55.6±0.1	10
D (C-8)/ACN	53.6±0.3	15
" /MeOH	54.6±0.6	6
E (C-18)/ACN	52.6±0.3	15

*Average $E_T(30)$ polarity where retention of alkylbenzenes is equivalent, obtained from intersection points of log k' versus $E_T(30)$ lines. Confidence intervals are based on t statistic, n-1 degrees of freedom.

155

polarity for equivalent retention is always greater when methanol is used as the organic modifier for a given column. For columns B, C, and D, this difference is statistically significant at the 95% confidence level.

In addition, as the length of the bonded phase chain increases, a mobile phase that is less polar (stronger) is required to achieve equivalent retention. This trend makes physical sense, in that as the length of the carbon chain is increased, the stationary phase surface behaves more like an alkane, and thus one would need a less polar mobile phase (hence stronger) to achieve equivalence of polarity. Moreover, the volume of stationary phase (V_s) is increased by lengthening the carbon chain, also leading to increased retention.

It is also interesting to note that in methanol/water systems, this point of equivalent retention is difficult to reach, since the $E_T(30)$ polarity of pure methanol is 55.6 kcal/mole, while with acetonitrile this is easily done, i.e., $E_T(30)$ = 45.6 kcal/mole for pure acetonitrile.

Therefore, these results suggest that the effective polarity of the stationary phase is different when in the presence of the two organic modifiers and that it is intermediate in polarity between methanol and acetonitrile.

Application of These Results

In Snyder's formulation of solvent P' values (Snyder, 1978), it was stated that the effective P' value for a mixture of two solvents, A and B, is given by

$$P'' = \phi_a \cdot P'_a + \phi_b \cdot P'_b \qquad (5\text{-}6)$$

where ϕ is the volume fraction of the two components. Since the two volume fractions must add up to one, the P'' is then given by

$$P'' = (1-\phi_b) \cdot P'_a + \phi_b \cdot P'_b \qquad (5\text{-}7)$$

Also, in terms of chromatographic retention of a given solute in two mobile phases of different P' values, the following expression can be written:

$$\log k'_b - \log k'_a = (P'_b - P'_a)/2 \qquad (5\text{-}8)$$

The constant "2" in equation 5-8 has no fundamental basis other than the observation by Snyder and Kirkland that ". . . a change in P' by two units causes (very roughly) a 10-fold change in k' values . . ." (Snyder and Kirkland, 1979, p. 258). Both of these equations are consistent with the statement "log k' will vary in a linear manner with the volume fraction of the organic modifier." As has been

discussed, this assumption is generally not warranted. Moreover, the magnitude of P' values for methanol and acetonitrile implies that k' values should actually increase when going from methanol to acetonitrile (as discussed in Chapter II). In view of these problems, better results could be obtained by using the $E_T(30)$ polarity values for the solvent mixtures themselves. This leads to the following expression:

$$\log k_2' - \log k_1' = [E_T^2(30) - E_T^1(30)] \cdot s \qquad (5\text{-}9)$$

where s is a constant for a given solute and column. Of course, this is precisely what one is doing when plotting log k' versus $E_T(30)$ polarity values. In this case the slope of such a line would correspond to the parameter s.

There is another way in which this approach may be extended, by using the equation that Langhals proposed to account for the variation in $E_T(30)$ polarity as a function of solvent mixture composition (see also the discussion of this equation in Chapter I).

The "Langhals equation" relates the $E_T(30)$ polarity value to the molar concentration (Cp) of the most polar component in a binary mixture as shown below:

$$E_T(30) = E_d \cdot \ln [(C_p/C^*) + 1] + E_T^0(30) \qquad (5\text{-}10)$$

In the above equation, E_d, $C*$, and $E_T^o(30)$ are constants, and have been reported for 46 solvent systems (Langhals, 1982a). Thus, it is possible to derive an expression relating a change in the concentration of organic modifier to a change in the log k' value for a given compound. First, the concentration of water in moles per liter is changed to percent organic modifier by

$$Cp = (100 - \% \text{ organic}) \cdot a \qquad (5-11)$$

where a is a constant incorporating the density and molecular weight of water. At 20^oC, this constant has a value of 0.5541. Substituting equation 5-11 into 5-10 leads to the following equation for a change in $E_T(30)$ polarity with changing percentage of organic modifier:

$$\Delta E_T(30) = E_d \cdot \ln\left(\frac{[(100 - \% \text{ organic}_1) \cdot a] + C*}{[(100 - \% \text{ organic}_2) \cdot a] + C*}\right) \qquad (5-12)$$

The change in log k' can then be calculated by using equation 5-9.

Equation 5-9 is also quite useful for predicting changes in retention when the organic modifier is changed between acetonitrile and methanol. As discussed previously in this chapter, there is evidence that the slope of log k' versus $E_T(30)$ polarity varies in a systematic manner in the two solvent systems examined here. Therefore, if the slope

is known for one of the solvent systems, the ratio of the slopes for the column can then be used to calculate the slope for the other solvent system. Then using equations 5-9 and 5-12, the change in log k' can be predicted for the second solvent system without making any additional measurements.

Herein lies one of the great strengths of these polarity measurements, in that changes in chromatographic separations can be predicted with minimal need for collection of experimental data. For a given column, only three experiments need be done for a prediction to be made with regard to the retention in the two solvent systems and at various concentrations. These three experiments can be summarized as follows:

1) measure k' in solvent system A at a low concentration of organic modifier

2) measure k' in solvent system A at a high concentration of organic modifier

3) measure k' in solvent system B at any single concentration of organic modifier.

Experiments 1 and 2 serve to establish the slope of $E_T(30)$ versus organic modifier (parameter "s" in equation 5-9). Experiment 3 establishes one point on the line for system B, which when combined with the slope ratio for the two systems and using equation 5-12 allows prediction of

160

changes in retention as a function of organic modifier and its concentration.

In conclusion, the predictive ability of these types of experiments can be applied in a number of ways; optimization of separation conditions for complex mixtures can be undertaken with a minimum amount of initial experimental work, involving as little as three retention measurements for each compound.

Interfacial Tension Effects

One aspect of chromatographic retention that will also be discussed briefly is that of the role of interfacial tension in solute retention. A number of investigators have shown that retention may be viewed as a result of the interfacial tension between the mobile and stationary phases. Therefore, the surface tension of the mobile phase is likely to play a major role in determining solute retention. As the surface tension of the mobile phase is lowered, the distribution coefficient of the solute should also be lowered, leading to a decrease in retention. Of course, since water has a very high surface tension with respect to organic modifiers, the observed decrease in retention is consistent with the predicted effect. In Figures 5-3 through 5-6, the variation in surface tension in binary methanol/water and acetonitrile/water mixtures is
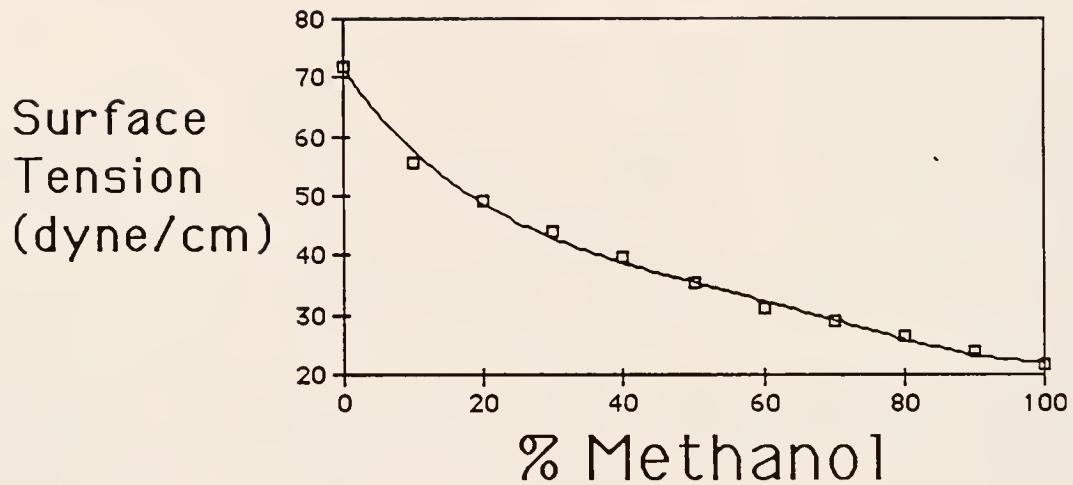
Figure 5-3. Variation in surface tension as a function of percent methanol.
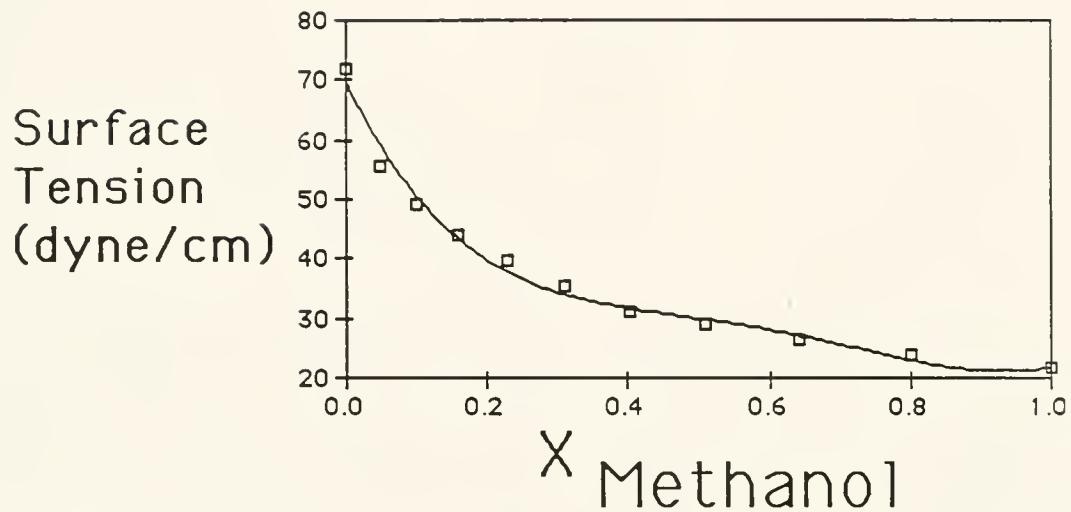
Figure 5-4. Variation in surface tension as a function of mole fraction of methanol.
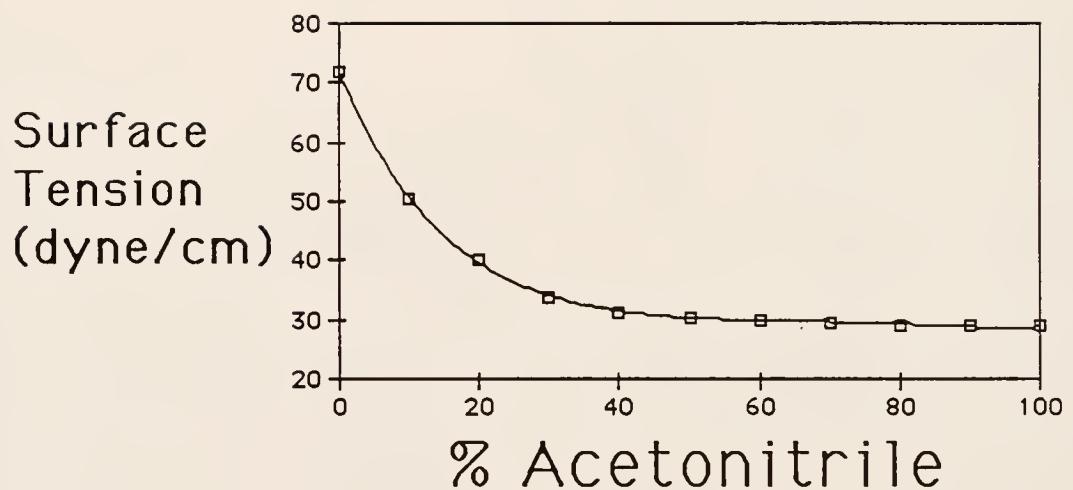
Figure 5-5. Variation in surface tension as a function of percent acetonitrile.
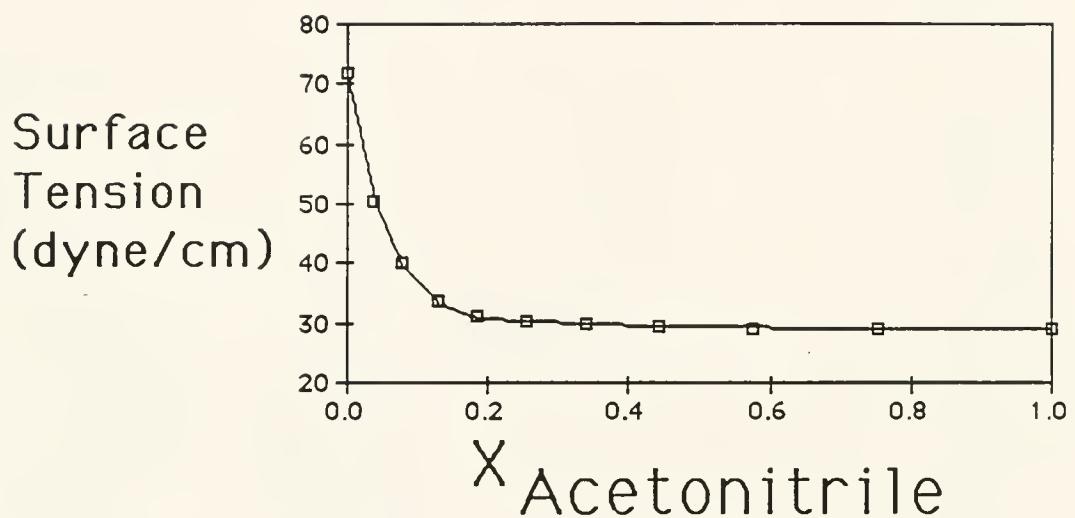
Figure 5-6.   Variation in surface tension as a function of mole fraction of acetonitrile.

shown with respect to volume percent and mole fraction. In both cases, the surface tension is a highly nonlinear function of composition. In the case of acetonitrile, there is a rapid drop at the lower concentrations, followed by a plateau, while methanol exhibits a steady, almost linear decrease at higher concentrations.

The effect of interfacial tension in solute retention has been illustrated by Deming and co-workers (Stranahan and Deming, 1982; Wu and Deming, 1985), who derived a four-parameter thermodynamic model of solute retention. In particular, the effect of surfactant concentration was examined; it was shown that capacity factors of uncharged solutes could be related to the concentration of a surfactant in the mobile phase by

$$k_i = k_{o,i} \cdot (1 + c_j/b_j)^{-b} \qquad (5\text{-}13)$$

where c_j is the concentration of the surfactant, and b_j and b are constants that are related to the ability of the surfactant to lower the interfacial tension of the system. Taking the logarithm of equation 5-13 then leads to the following expression:

$$\ln k_i = \ln k_{o,i} + -b \cdot \ln(1 + c_j/b_j) \qquad (5\text{-}14)$$

What is especially interesting is that the previously discussed Langhals equation is of the exact same form (see equation 5-10; this equation is also discussed in Chapter I). In the Langhals equation, the correlary to the c_j surfactant concentration term of equation 5-14 is c_p, which is the concentration of the most polar component of a binary mixture. Since there is such a strong correlation between $E_T(30)$ polarity and retention, it is worthwhile to construct plots of $E_T(30)$ versus surface tension in the two organic modifier systems.

In Figures 5-7 and 5-8, the surface tension for binary mixtures is compared with the $E_T(30)$ polarity for the methanol and acetonitrile systems, respectively. Data points for 90 and 100% acetonitrile have been omitted from Figure 5-8, in order to avoid compressing the axes (the $E_T(30)$ polarity changes by >10 in this range). For methanol/water mixtures, the relationship is nearly linear if 100% water is excluded ($E_T(30)$ = 63.1 kcal/mole), while in acetonitrile/water mixtures strong curvature is noted.

Suggestions for Future Research

A number of potential extensions of this research have been identified. The present research has dealt with the retention and selectivity of solutes in reversed phase systems, since it is the most widely used LC technique.
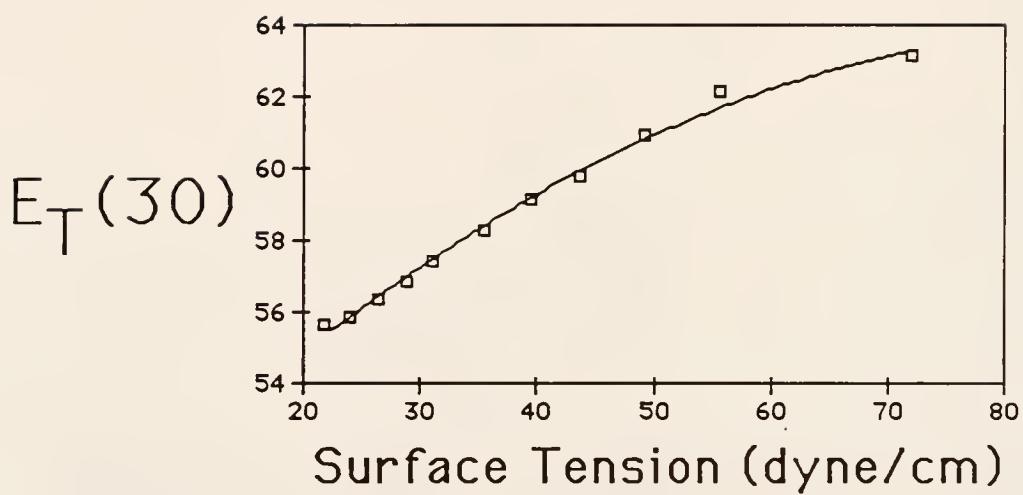
Figure 5-7. Comparison between surface tension and $E_T(30)$ polarity for methanol/water mixtures.

168
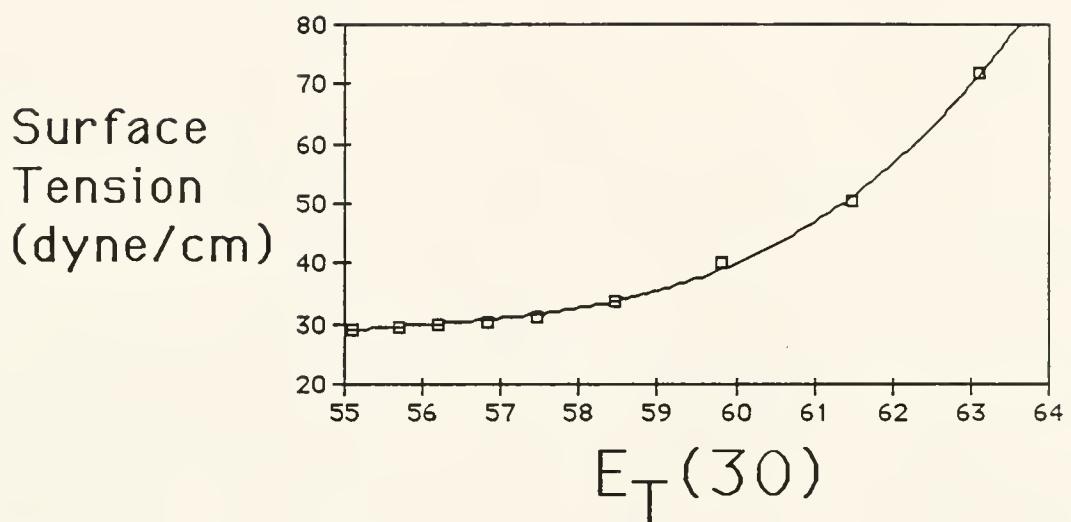
Figure 5-8. Comparison between surface tension and $E_T(30)$ polarity for acetonitrile/water mixtures (0-80% v/v).

However, there are still many separations for which normal phase chromatography is the method of choice. This is especially true for highly polar compounds which are poorly retained by typical bonded phases. Normal phase liquid chromatography (NPLC) involves the use of either silica or bonded phases with polar groups such as nitrile or amino groups. Typical mobile phases are nonaqueous binary or ternary solvent mixtures (for example, hexane/chloroform or heptane/ethanol), whose strength is adjusted by varying the proportion of the components.

It should, therefore, be possible to determine the $E_T(30)$ polarity of these mixtures as has been done with the water/organic systems described in this dissertation and to then compare these measurements with chromatographic retention, as well as Snyder's ε^o eluent strength parameters for NPLC solvents. A potential problem with NPLC mobile phases (and one that can be solved) is the low solubility of the ET-30 in pure nonpolar solvents such as hexane or heptane. As described in Chapter I, the use of the penta (tert-butyl) derivative of ET-30 allows one to measure the $E_T(30)$ polarity of such solvents. Thus, by using this more lipophilic betaine dye, one may measure the effective $E_T(30)$ polarity of the mixture, since there is a linear correlation between its absorption maximum and that of ET-30 in solvents of mutual solubility (Reichardt and Harbusch-Gornert, 1983).

Another potentially fruitful area is that of examining the surface polarity of stationary phases with the ET-30. Pyrene has been used to a large extent as a probe of the surface polarity (see Chapter I), but its use at high organic modifier concentrations has been hindered by solubility in the mobile phase. On the other hand, the ET-30 dye may be an ideal probe molecule for the surface polarity measurement of bonded phases. Attempts to measure the capacity factor of the ET-30 dye were unsuccessful; it was found that ET-30 is completely retained by a reversed phase column packing, even with a mobile phase of 100% acetonitrile. Thus, it is likely that this dye would be ideally suited as a probe molecule, since it has a high affinity for the stationary phase and appears to remain sorbed to the packing even in the presence of 100% organic modifier. It should be possible to sorb the dye onto a reversed phase packing and then to measure its (UV/VIS) diffuse reflectance spectrum, using the packing with no sorbed ET-30 as the reference. It should also be possible to obtain these spectra in the presence of various concentrations of organic modifier and thus obtain surface polarity data that cannot be obtained with pyrene as the probe. Using this approach, one could then compare the variation in stationary phase polarity as a function of organic modifier concentration with that of the mobile phase. That is, the same probe molecule would be used to

171

measure the polarity of the two phases, helping to minimize specific solvent association effects for the ET-30 dye.

A potential problem is that of overloading the column packing, in which case the results would not be indicative of the true condition of the stationary phase. In fact, this problem is implied by the work published by Lindley et al. (1985), who carried out experiments of this type with dry samples of silica (discussed in Chapter I). The $E_T(30)$ polarity was found to be a function of its concentration, which implied that more than a monolayer of the dye had been applied and/or dimerization had occurred.

The solvatochromic comparisons recently reported by Sadek et al. (1985b; equation 3-7) included no solutes with significant hydrogen bond donating ability (α value of zero). The α values are measured by the enhanced solvatochromism of ET-30 with respect to 4-nitroanisole. It would be worthwhile to attempt correlations of the type used by Sadek and Carr, with the inclusion of the α value as a parameter in equation 3-7 (in addition to those already used--molar volume, β, and π^*). The α values may be found in the literature (Kamlet et al., 1983), or experimentally determined.

To further clarify the meaning of the observed slope of log k' versus $E_T(30)$ polarity in the different solvent systems, it would be useful to measure retention and $E_T(30)$ polarity for a homologous series of organic modifiers.

That is, by using a homologous series, such as methanol, ethanol, n-propanol, etc., one could better interpret the different slopes in log k' versus $E_T(30)$ polarity, since these modifiers would be expected to solvate the stationary phase in direct proportion to their size. Therefore, any change in the slope would add further evidence to the argument that a change in the nature of the stationary phase is actually being measured. Also, use of a homologous series as a mobile phase will limit specific solvent association effects with the $E_T(30)$.

In addition, ternary solvent systems could be explored in the same manner as the binary systems previously discussed in this dissertation. For a system such as $MeOH/ACN/H_2O$, there should be a gradual change in the slopes of log k' versus $E_T(30)$ polarity as the ternary mixtures are varied between the two binary extremes. This would provide additional information about the solvation of the bonded phase alkyl chains.

Lastly, these results can be applied to gradient elution schemes, in which the strength of the mobile phase is changed during the separation. Snyder and Dolan have derived a number of useful equations in which the optimum gradient can be selected (Dolan et al., 1979; Snyder et al., 1979). However, in the derivations of what constitutes an optimal gradient, it is assumed that log k' values vary as a linear function of the organic modifier

concentration. Since the $E_T(30)$ polarity values represent a better measure of mobile phase strength, it should be possible to substitute the concentration dependence of $E_T(30)$ values for the portion of these equations in which the function log "k' = f(ϕ)" is incorporated. This function could be obtained from either a polynomial least-squares fit of $E_T(30)$ versus percent organic modifier, or from the Langhals equation (Langhals, 1982a). In this manner, it should be possible to derive more optimum gradients which reflect the true variation in retention as a function of the strength of the mobile phase.

APPENDIX A
CHROMATOGRAPHIC RETENTION AND
SELECTIVITY DATA

Reference: This Work
Column: Ultrasphere ODS
Mobile Phase: acetonitrile/water

Compound				k' at % ACN				
	10	20	30	40	50	60	70	80
2-Nitroaniline	15.950	7.178	3.664	2.279	1.377	0.592		
4-Nitroaniline	7.917	3.940	2.105	1.417	0.921	0.872		
4-Nitroanisole	48.435	19.218	8.478	4.491	2.415	1.479	0.891	0.497
4-Nitrophenol	12.452	5.263	2.490	1.547	0.929	0.601		

Reference: This Work
Column: Hamilton PRP-1

Mobile Phase: methanol/water

	k' for nitroalkanes (NCx)*				
%MeOH	NC1	NC2	NC3	NC4	NC6
0	3.2192	21.5225			
10	2.6168	15.3288	95.7718	---	---
20	2.1532	10.0736	51.9280	---	---
30	1.7178	6.7628	29.4204	---	---
40	1.3123	4.4429	15.7417	---	---
50	1.0308	3.0691	8.6171	---	---
60	0.7455	1.8679	4.2665	10.6629	---
70	0.5728	1.2335	2.4047	5.0586	---
80	0.6367	1.0495	1.6877	2.9039	8.3844
90	0.5090	0.7267	0.9921	1.4444	---
100	0.4001	0.5015	0.5856	0.7230	1.0758

Mobile Phase: acetonitrile/water

	k' for nitroalkanes (NCx)				
%ACN	NC1	NC2	NC3	NC4	NC6
0	4.5122	31.5203	---	---	---
10	2.1382	7.5122	29.2439	---	---
20	1.5203	4.1057	11.8130	34.8537	---
30	1.1138	2.5528	5.9268	13.7804	---
40	0.7642	1.4797	3.2520	6.3984	23.1463
50	0.6992	1.1789	2.0163	3.4472	9.4553
60	0.5447	0.8374	1.2850	1.9756	4.4309
70	0.4285	0.6081	0.8659	1.2358	2.4545
80	0.3610	0.4675	0.6154	0.8138	0.6041
90	0.2138	0.2813	0.3455	0.4374	0.7098
100	0.1756	0.2089	---	---	---

*NCx denotes nitroalkane of carbon chain length x (i.e., NC1 = nitromethane).

Reference: Woodburn (1985)
Column: Sepralyte C-2
Mobile Phase: methanol/water

Compound	log k' at % methanol			
	35	40	50	60
Biphenyl	1.3971	1.1868	0.7372	0.1761
Napthalene	0.9912	0.8276	0.4637	-0.0395
Phenanthrene	1.6086	1.3616	0.8479	0.2389
Anthracene	1.6789	1.4328	0.9034	0.2815
Pyrene	1.9116	1.6294	1.0400	0.3738
Chrysene		1.9533	1.2737	0.5334
Fluoranthene	1.9072	1.6205	1.0293	0.3673
n-Butylbenzene	1.6746	1.4560	0.9782	0.3863
Benzene	0.3335	0.2605	0.0467	-0.3256
Toluene	0.6222	0.5206	0.2569	-0.1586
Ethylbenzene	0.9229	0.7892	0.4713	0.0071
n-Propylbenzene	1.2816	1.1099	0.7216	0.1984
p-Xylene	0.9430	0.8265	0.4828	0.0164
o-Xylene	0.8784	0.7426	0.4326	-0.0264
m-Diethylbenzene	1.5479	1.3282	0.8950	0.3409
1,2,4-Trimethyl-benzene	1.2103	1.0338	0.6570	0.1501
Fluorobenzene	0.4452	0.3560	0.1162	-0.2730
Chlorobenzene	0.7064	0.5894	0.3023	-0.1330
Bromobenzene	0.7815	0.6582	0.3478	-0.1140
Iodobenzene	0.9181	0.7748	0.4339	-0.0397
Nitrobenzene	0.3026	0.2592	0.0169	-0.3736

Reference: Woodburn (1985)
Column: Sepralyte C-4
Mobile Phase: methanol

	log k' at % methanol				
Compound	40	50	60	70	75
Biphenyl		1.1613	0.6889	0.2201	-0.0104
Napthalene	1.2568	0.8609	0.4717	0.0567	-0.1395
Phenanthrene		1.2757	0.7607	0.2562	0.029
Anthracene		1.3223	0.8007	0.2371	0.0398
Pyrene		1.7086	0.9154	0.3701	0.1255
Chrysene		1.7086	1.0714	0.4659	0.1956
n-Butylbenzene		1.4647	0.9423	0.4329	0.1891
n-Hexylbenzene			1.3944	0.7591	0.4574
Benzene	0.6405	0.4108	0.1453	-0.1775	-0.3159
Toluene	0.938	0.6486	0.3209	-0.0334	-0.1963
Ethylbenzene	1.2581	0.8944	0.5119	0.1111	-0.0733
n-Propylbenzene		1.1759	0.7272	0.2717	0.0562
p-Xylene	1.2465	0.8986	0.5108	0.1171	-0.0739
o-Xylene		0.0842	0.4752	0.0931	-0.0836
m-Diethylbenzene		1.3808	0.9014	0.3942	0.1587
1,2,4-Trimethyl-benzene	1.5123	1.0948	0.6709	0.2376	0.0303
Fluorobenzene	0.7250	0.4684	0.6709	-0.1616	-0.3189
Chlorobenzene	0.9924	0.6852	0.3437	-0.0266	-0.1986
Bromobenzene	1.0677	0.7468	0.3966	0.0090	-0.1783
Iodobenzene	1.2172	0.8445	0.4621	0.0607	-0.1254
Nitrobenzene	0.6050	0.3437	0.0594	-0.2689	-0.4083

179

Reference: Woodburn (1985)
Column: Sepralyte C-8, 5 cm
Mobile Phase: methanol/water

Compound	log k' at % methanol			
	50	60	70	80
Anthracene	1.7629	1.1905	0.6736	0.1941
Pyrene	2.0105	1.3866	0.8446	0.3485
Chrysene	---	1.6004	0.9903	0.4377
n-Butylbenzene	1.7929	1.2241	1.7131	0.2177
n-Hexylbenzene	---	1.7403	1.0989	0.4962
Benzene	0.5736	0.2782	-0.0215	-0.3254
Toluene	0.8732	0.5178	0.1678	-0.1839
Ethylbenzene	1.1483	0.7330	0.3323	-0.0730
n-Propylbenzene	1.4672	0.9799	0.5194	0.0714
p-Xylene	1.1634	0.7602	0.3591	-0.0322
o-Xylene	1.1109	0.7087	0.3270	-0.0566
m-Diethylbenzene	1.7098	1.1783	0.6760	0.1952
1,2,4-Trimethyl-benzene	1.4139	0.9617	0.5201	0.1444
Fluorobenzene	0.6263	0.3049	-0.0125	-0.3444
Chlorobenzene	0.8981	0.5346	0.1678	-0.1892
Bromobenzene	0.9763	0.5903	0.2151	-0.1486
Iodobenzene	1.1008	0.6951	0.2957	-0.0875
Nitrobenzene	0.4821	0.1810	-0.1163	-0.4178

Reference: Woodburn (1985)
Column: Sepralyte C-2
Mobile Phase: acetonitrile/water

	log k' at % acetonitrile			
Compound	25	30	40	50
Biphenyl	1.5805	1.3616	0.9107	0.4245
Napthalene	1.2347	1.0705	0.7082	0.2395
Phenanthrene	1.7216	1.4693	0.9716	0.4665
Anthracene	1.7826	1.5675	1.0104	0.4846
Pyrene	1.9169	1.6252	1.0755	0.5326
Chrysene	---	1.8772	1.2435	0.6456
Fluoranthene	1.6431	1.0870	0.5433	1.9350
n-Butylbenzene	1.7942	1.5516	1.0754	0.5717
Benzene	0.6590	0.6035	0.3890	0.0830
Toluene	0.9252	0.8211	0.5519	0.1966
Ethylbenzene	1.1980	1.0569	0.7211	0.3137
n-Propylbenzene	1.4949	1.3148	0.9042	0.4445
p-Xylene	1.1754	1.0463	0.7238	0.3067
o-Xylene	1.1327	1.0042	0.6810	0.2849
m-Diethylbenzene	1.7128	1.4997	1.0353	0.5251
1,2,4-Trimethyl- benzene	1.4034	1.2165	0.8319	0.4124
Fluorobenzene	0.7588	0.6782	0.4404	0.1082
Chlorobenzene	0.9660	0.8552	0.5865	0.2056
Bromobenzene	1.0315	0.8991	0.6024	0.2264
Iodobenzene	1.1574	1.0129	0.6767	0.2790
Nitrobenzene	0.6587	0.5843	0.3561	0.0460

181

Reference: Woodburn (1985)
Column: Sepralyte C-4
Mobile Phase: acetonitrile/water

| Compound | log k' at % acetonitrile | | | |
	30	40	50	60
Biphenyl	1.5463	1.0381	0.6572	0.3197
Napthalene	1.2330	0.8206	0.4993	0.2059
Phenanthrene	1.6648	1.0980	0.6953	0.3801
Anthracene	1.7007	1.1354	0.7255	0.3714
Pyrene	1.8270	1.2077	0.7798	0.4130
Chrysene	2.0831	1.3748	0.8934	0.4936
Fluoranthrene	1.8203	1.2151	0.7818	0.4122
n-Butylbenzene	1.8148	1.2576	0.7818	0.4122
n-Hexylbenzene	---	1.6561	1.1506	0.7244
Benzene	0.7452	0.4861	0.2643	0.0347
Toluene	0.9803	0.6719	0.3996	0.1412
Ethylbenzene	1.2399	0.8631	0.5423	0.2510
n-Propylbenzene	1.5225	1.0694	0.6896	0.3714
p-Xylene	1.2344	0.8418	0.5336	0.2453
o-Xylene	1.1921	0.8101	0.5056	0.2231
m-Diethylbenzene	1.7286	1.2216	0.8135	0.4785
1,2,4-Trimethyl-benzene	1.4300	1.2216	0.6401	0.3404
Fluorobenzene	0.8103	0.5271	0.2867	0.0476
Chlorobenzene	1.0022	0.6751	0.4003	0.1339
Bromobenzene	1.0732	0.7199	0.4334	0.1597
Iodobenzene	1.1845	0.7995	0.4929	0.2121
Nitrobenzene	0.6840	0.4229	0.1919	-0.0291

Reference: Woodburn (1985)
Column: Sepralyte C-8
Mobile Phase: acetonitrile

log k' at % acetonitrile

Compound	30	40	50	60	65	80
Biphenyl	1.8791	1.3100	0.8969	0.4778	0.3277	-0.0375
Napthalene	1.5346	1.0692	0.7220	0.3510	0.2169	-0.1091
Phenanthrene		1.4679	1.0000	0.5769	0.4229	0.0361
Anthracene		1.4878	1.0400	0.6040	0.4483	0.0497
Pyrene		1.6562	1.1580	0.7193	0.5604	0.1646
Chrysene		1.8531	1.2986	0.8193	0.6443	0.2006
Fluoranthrene		1.6384	1.1402	0.6882	0.5265	0.1210
n-Butylbenzene		1.5767	1.1105	0.6716	0.5092	0.1177
n-Hexylbenzene		2.0444	1.4855	0.9692	0.7819	0.3242
Benzene	0.6637	0.4213	0.1172	0.0121	-0.2418	0.9515
Toluene	0.8929	0.5896	0.2413	0.1325	-0.1555	1.2307
Ethylbenzene	1.5186	1.1057	0.7544	0.3784	0.2446	-0.0809
n-Propylbenzene	1.8364	1.3437	0.9386	0.5222	0.3766	-0.0167
p-Xylene	1.5235	1.0932	0.7457	0.3809	0.2525	-0.0719
o-Xylene	1.4667	1.0695	0.7154	0.3582	0.2279	-0.0971
m-Diethylbenzene	2.0636	1.5178	1.0768	0.6434	0.4865	0.0918
1,2,4-Trimethyl-benzene	1.7523	1.2679	0.8851	0.4925	0.3565	0.0112
Fluorobenzene	1.0245	0.7037	0.4439	0.1249	0.0121	-0.2406
Chlorobenzene	1.2773	0.9011	0.5891	0.2534	0.1325	-0.1484
Bromobenzene	1.3471	0.9518	0.6410	0.2886	0.1699	-0.1272
Iodobenzene	1.4961	1.0642	0.7205	0.3582	0.2315	-0.1000
Nitrobenzene	0.9093	0.6066	0.3447	0.0352	-0.0659	-0.3156

Reference: Woodburn (1985)
Column: Sepralyte C-18
Mobile Phase: acetonitrile/water

Compound	log k' at % acetonitrile			
	50	55	60	70
Biphenyl	1.0927	0.9084	0.7525	0.4691
Napthalene	0.8931	0.7295	0.5935	0.3383
Phenanthrene	1.2411	1.0467	0.8804	0.5906
Anthracene	1.2903	1.0922	0.9276	0.6283
Pyrene	1.4429	1.2395	1.0708	0.7673
Chrysene	1.6263	1.3996	1.2128	0.8803
n-Butylbenzene	1.3610	1.1659	0.9991	0.6933
n-Hexylbenzene	1.8049	1.5739	1.3778	1.0242
Benzene	0.5283	0.4124	0.3061	0.0965
Toluene	0.7362	0.5918	0.4703	0.2449
Ethylbenzene	0.9278	0.7655	0.6279	0.3746
n-Propylbenzene	1.1479	0.9661	0.8136	0.5349
p-Xylene	0.9407	0.7826	0.6410	0.3930
o-Xylene	0.8971	0.7428	0.6038	0.3583
m-Diethylbenzene	1.3018	1.1108	0.9479	0.6495
1,2,4,-Trimethyl- benzene	1.1080	0.9371	0.7860	0.5189
Fluorobenzene	0.5408	0.4113	0.2953	0.0810
Chlorobenzene				
Bromobenzene	0.2805	0.5174	0.6481	0.7942
Iodobenzene	0.8980	0.7416	0.6043	0.3583
Nitrobenzene	0.4113	0.2872	0.1746	-0.0347

Reference: Jandera (1985)
Column: Silasorb C-8
Mobile Phases: acetonitrile/water

Compound	log k' at % acetonitrile			
	50	60	70	80
Acetophenone	-0.1249	-0.301	-0.4949	-0.699
Anisole	0.0792	-0.1249	-0.3979	-0.6021
Benzaldehyde	-0.1135	-0.3098	-0.5376	-0.7696
Benzonitrile	-0.0605	-0.2596	-0.5086	-0.7212
Benzophenone	0.3096	0.0253	-0.2441	-0.5086
Benzotrichloride	0.5999	0.2672	-0.0044	-0.301
Bromobenzene	0.3075	0.0645	-0.2076	-0.4437
n-Butyl bromide	0.3655	0.0934	-0.1611	-0.3979
n-Butyl phenyl- carbamate	0.2672	-0.0088	-0.2757	-0.5528
Chlorobenzene	0.2788	0.0212	-0.2366	-0.4815
Chlorobromuron	0.2553	-0.0132	-0.284	-0.5376
m-Cresol	-0.8361	-0.699	-0.4202	-0.2291
o-Cresol	-0.2147	-0.4202	-0.6198	-0.8361
p-Cresol	-0.1675	-0.3979	-0.6198	-0.8861
Di-n-butyl ether	0.5647	0.2718	0.0044	-0.2291
Ether benzoate	0.2989	0.0607	-0.2218	-0.4685
n-Heptane	1.0141	0.6749	0.3856	0.0792
Linuron	0.2253	-0.041	-0.3098	-0.5686
Methyl benzoate	0.0414	-0.1487	-0.3872	-0.6198
Nitrobenzene	0.0212	-0.2007	-0.4437	-0.6576
n-Octane	1.2095	0.8331	0.5172	0.1987
Phenetole	0.1847	-0.0044	-0.3188	-0.5086
Phenol	-0.3279	-0.5528	-0.699	-1.0969
Phenyl acetate	-0.1135	-0.284	-0.5086	-0.7212
n-Propyl phenyl ether	0.3284	0.0828	-0.1871	-0.4559
Styrene	0.3032	0.0374	-0.2366	-0.4685

Reference: Jandera (1985)
Column: Silasorb C8
Mobile Phase: methanol/water

| | log k' at % methanol | | | |
Compound	60	70	80	90
Acetophenone	-0.2076	-0.4949	-0.7447	-1.000
Anisole	-0.0223	-0.301	-0.585	-0.9208
Benzaldehyde	-0.284	-0.5229	-0.7959	-1.1549
Benzonitrile	-0.2518	-0.5376	-0.7959	-1.0969
Benzophenone	0.2625	-0.1192	-0.4685	-0.8239
Benzotrichloride	0.6128	0.1614	-0.2366	-0.6383
Bromobenzene	0.281	-0.0655	-0.4089	-0.7696
n-Butyl bromide	0.2833	-0.0555	-0.3872	-0.7696
n-Butyl phenyl-carbamate	0.1732	-0.1938	-0.6021	-1.000
Chlorobenzene	0.2175	-0.1135	-0.4437	-0.7959
Chlorobromuron	0.248	-0.1367	-0.4815	-0.9208
m-Cresol	-0.2291	-0.4202	-0.699	-0.8861
o-Cresol	-0.3279	-0.6198	-0.8861	-1.301
p-Cresol	-0.1675	-0.3979	-0.6198	-0.8861
Di-n-butyl ether	0.5514	0.1239	-0.2366	-0.6198
Ethyl benzoate	0.2695	-0.1024	-0.4559	-0.7959
n-Heptane	1.1424	0.6405	0.1523	-0.3468
Linuron	0.2095	-0.1805	-0.5376	-0.8861
Methyl benzoate	0.0086	-0.3188	-0.6198	-0.9208
Nitrobenzene	-0.1249	-0.3979	-0.6778	-1.0458
Phenetole	0.1461	-0.2007	-0.4815	-0.8239
Phenol	-0.5376	-0.7212	-1.000	-1.5229
Phenyl acetate	-0.2147	-0.4949	-0.7447	-1.000
n-Propyl phenyl ether	0.2695	-0.0862	-0.4318	-0.7959
Styrene	0.2601	-0.0862	-0.4202	-0.7696

Reference: Hanai and Hubert (1983)
Column: Unisil Q C18
Mobile Phase: Acetonitrile/water

log k' at % acetonitrile

Compound	90	80	70	60	50	40	30	20	10
Phenol	-0.052	-0.006	0.082	0.168	0.304	0.481	0.695	0.951	1.293
2-Methylphenol	0	0.064	0.184	0.298	0.481	0.704	0.985	1.327	---
3-Methylphenol	-0.018	0.047	0.156	0.263	0.441	0.661	0.935	1.291	---
4-Methylphenol	-0.117	0.052	0.160	0.266	0.440	0.662	0.936	1.292	1.729
2,3-Dimethylphenol	0.039	0.133	0.260	0.400	0.610	0.877	1.216	---	---
2,4-Dimethylphenol	0.044	0.176	0.268	0.413	0.624	0.893	1.236	---	---
2,5-Dimethylphenol	0.039	0.129	0.263	0.409	0.613	0.890	1.232	---	---
2,6-Dimethylphenol	0.059	0.158	0.297	0.442	0.646	0.923	1.249	---	---
3,4-Dimethylphenol	0.017	0.103	0.213	0.350	0.537	0.806	1.135	1.567	---
3,5-Dimethylphenol	0.023	0.114	0.230	0.372	0.570	0.845	1.184	1.621	---
2,3,5-Trimethyl-phenol	0.084	0.199	0.349	0.520	0.751	1.071	1.468	---	---
2,3,6-Trimethyl-phenol	0.107	0.229	0.386	0.558	0.789	1.105	1.484	---	---
2,4,6-Trimethyl-phenol	0.116	0.240	0.400	0.574	0.807	1.126	1.515	---	---
2,3,5,6-Tetra-methylphenol	0.211	0.358	0.534	0.704	0.935	1.244	---	---	---
2-Ethylphenol	0.028	0.121	0.244	0.387	0.587	0.870	1.221	1.675	---
3-Ethylphenol	0.023	0.109	0.234	0.381	0.581	0.861	1.208	1.648	---
4-Ethylphenol	0.031	0.116	1.244	0.384	0.587	0.870	1.221	1.665	---
2-Chlorophenol	-0.003	0.067	0.172	0.302	0.480	0.724	1.021	1.397	---
3-Chlorophenol	0	0.089	0.209	0.349	0.538	0.809	1.146	1.553	---
4-Chlorophenol	0.003	0.072	0.191	0.332	0.514	0.776	1.110	1.530	---
2,3-Dichlorophenol	0.047	0.144	0.280	0.448	0.670	0.968	1.393	---	---
2,4-Dichlorophenol	0.074	0.178	0.329	0.500	0.727	1.049	1.467	---	---

Reference: Hanai and Hubert (1983)--continued.
Column: Unisil Q C18
Mobile Phase: Acetonitrile/water

log k' at % acetonitrile

Compound	10	20	30	40	50	60	70	80	90
2,5-Dichlorophenol	---	---	1.453	1.046	0.724	0.488	0.310	0.162	0.054
2,6-Dichlorophenol	---	---	1.336	0.975	0.684	0.467	0.302	0.162	0.057
3,4-Dichlorophenol	---	---	1.497	1.061	0.731	0.493	0.318	0.166	0.062
3,5-Dichlorophenol	---	---	1.644	1.193	0.841	0.585	0.393	0.225	0.109
2,3,4-Trichlorophenol	---	---	---	1.268	0.891	0.617	0.423	0.247	0.131
2,3,5-Trichlorophenol	---	---	1.466	1.068	0.755	0.526	0.351	0.197	0.093
2,3,6-Trichlorophenol	---	---	---	1.247	0.893	0.626	0.432	0.258	0.131
2,4,5-Trichlorophenol	---	---	---	1.340	0.958	0.671	0.463	0.282	0.146
2,4,6-Trichlorophenol	---	---	---	1.331	0.971	0.696	0.495	0.314	0.174
3,4,5-Trichlorophenol	---	---	---	1.388	0.990	0.698	0.488	0.301	0.162
2,3,4,5-Tetra-chlorophenol	---	---	---	1.595	1.158	0.835	0.603	0.390	0.229
2,3,5,6-Tetra-chlorophenol	---	---	---	1.584	1.165	0.850	0.621	0.410	0.249
Pentachlorophenol	---	---	---	---	1.366	1.020	0.766	0.531	0.340
2-Chloro-5-methyl-phenol	---	---	1.290	0.925	0.638	0.425	0.263	0.131	0.039
4-Chloro-2-methyl-phenol	---	---	1.427	1.026	0.712	0.481	0.312	0.166	0.067
4-Chloro-3-methyl-phenol	---	---	1.355	0.962	0.660	0.442	0.279	0.147	0.052
2-Bromophenol	---	1.504	1.104	0.781	0.531	0.34	0.202	0.091	0.011
3-Bromophenol	---	1.664	1.222	0.868	0.592	0.386	0.234	0.114	0.025
4-Bromophenol	---	1.547	1.201	0.843	0.567	0.370	0.224	0.105	0.023
2,4-Dibromophenol	---	---	1.640	1.181	0.835	0.575	0.389	0.227	0.112
2,6-Dibromophenol	---	---	1.515	1.119	0.802	0.560	0.383	0.227	0.109

Reference: Hanai and Hubert (1983)--continued.
Column: Unisil Q C18
Mobile Phase: Acetonitrile/water

log k' at % acetonitrile

Compound	90	80	70	60	50	40	30	20	10
2-Nitrophenol	0.047	0.144	0.266	0.412	0.601	0.843	1.136	1.462	---
3-Nitrophenol	-0.059	0.009	0.105	0.224	0.391	0.618	0.906	1.266	---
4-Nitrophenol	-0.072	-0.012	0.072	0.191	0.351	0.579	0.860	1.210	1.635
2,4-Dinitrophenol	-0.046	0.047	0.156	0.302	0.504	0.762	1.081	1.444	---
2,5-Dinitrophenol	-0.042	0.054	0.174	0.332	0.542	0.814	1.134	1.435	---
2,6-Dinitrophenol	-0.042	0.052	0.170	0.322	0.525	0.779	1.067	1.368	---
3,4-Dinitrophenol	-0.069	-0.012	0.096	0.247	0.464	0.763	1.153	1.622	---
2-Hydroxyaceto-phenone	-0.033	0	0.033	0.98	0.195	0.332	0.518	0.792	1.232
4-tert-Butylphenol	0.105	0.232	0.392	0.585	0.851	1.214	---	---	---
4-Hydroxypropyl benzoate	0.023	0.112	0.235	0.390	0.611	0.924	1.350	---	---
4-Hydroxybutyl benzoate	0.074	0.187	0.344	0.532	0.801	1.171	1.679	---	---
4-Chloro-3,5-dimethylphenol	0.107	0.218	0.378	0.559	0.808	1.153	1.608	---	---
1,3-Dihydroxy-benzene	-0.155	-0.135	-0.100	-0.055	0.025	0.129	0.256	0.441	0.748
1,2-Dihydroxy-benzene	-0.112	-0.086	-0.049	0.020	0.103	0.222	0.375	0.591	0.904
1,4-Dihydroxy-benzene	-0.042	-0.155	-0.104	-0.094	-0.040	0.054	0.158	0.277	0.481
2-Hydroxy-naphthalene	0.023	0.116	0.234	0.389	0.603	0.905	1.294	---	---
1-Hydroxy-naphthalene	0.044	0.140	0.276	0.443	0.669	0.986	1.389	---	---
1-Hydroxy-2,4-dinitronaphtalene	0.121	0.265	0.450	0.653	0.946	1.314	---	---	---

Reference: Hanai and Hubert (1983)
Column: Hypersil ODS
Mobile Phase: Acetonitrile/water

Compound	log k' at % acetonitrile						
	80	70	60	50	40	30	20
4-Nitrophenol	-0.221	-0.153	0.002	0.160	0.373	0.667	1.006
2,4-Dinitrophenol	-0.158	-0.089	0.119	0.313	0.570	0.900	1.255
3-Bromophenol	-0.082	-0.021	0.188	0.385	0.659	1.025	1.470
4-Chloro-3-methylphenol	-0.065	0.016	0.242	0.454	0.752	1.156	---
2,5-Dichlorophenol	-0.051	0.048	0.292	0.525	0.842	1.256	---
4-Chloro-3,5-dimethyl-phenol	0.025	0.105	0.360	0.402	0.943	1.401	---
2,4,5-Trichlorophenol	0.080	0.176	0.470	0.746	1.131	---	---
2,3,4,5-Tetrachloro-phenol	0.179	0.296	0.630	0.943	1.387	---	---

Reference: Hanai and Hubert (1985)
Column: Hypersil ODS

k' at % acetonitrile

Compound	70	60	50	40	30	20	10
Aniline	0.53	0.67	0.91	1.35	2.09	3.51	6.98
N-Methylaniline	0.87	1.23	1.91	3.29	6.04	11.74	---
N-Ethylaniline	1.15	1.71	2.90	5.46	11.22	24.56	---
N-Butylaniline	2.15	3.75	7.72	19.06	---	---	---
N,N-Dimethylaniline	1.50	2.26	3.89	7.63	16.29	---	---
N,N-Diethylaniline	2.71	4.71	9.71	24.13	---	---	---
2-Methylaniline	0.67	0.89	1.31	2.09	3.67	7.25	16.95
3-Methylaniline	0.70	0.87	1.30	2.12	3.85	7.82	18.79
4-Methylaniline	0.67	0.87	1.27	2.07	3.77	7.69	18.79
2,4-Dimethylaniline	0.86	1.18	1.86	3.25	6.61	15.79	---
4-Methoxyaniline	0.44	0.49	0.69	0.99	1.59	2.95	7.06
2,4-Diethoxyaniline	0.92	1.32	2.22	4.24	9.61	27.19	---
2-Chloroaniline	0.84	1.18	1.90	3.41	6.76	14.32	---
3-Chloroaniline	0.76	1.06	1.73	3.15	6.50	14.79	---
4-Chloroaniline	0.74	1.01	1.60	2.84	5.82	13.33	---
2,5-Dichloroaniline	1.25	1.94	3.57	7.67	19.52	---	---
3,4-Dichloroaniline	1.01	1.52	2.72	5.69	14.55	---	---
4-Bromoaniline	0.80	1.13	1.83	3.40	7.29	17.82	---
2-Nitroaniline	0.67	0.91	1.41	2.56	5.09	11.22	27.94
3-Nitroaniline	0.58	0.77	1.22	2.10	3.98	8.07	17.54
4-Nitroaniline	0.48	0.64	0.99	1.69	3.17	6.53	14.80
Pyridine	0.61	0.64	0.77	1.01	1.46	2.45	5.64
2-Aminopyridine	0.28	0.22	0.24	0.28	0.38	0.59	1.28
3-Aminopyridine	0.32	0.31	0.35	0.44	0.61	1.02	2.40
4-Aminopyridine	---	---	---	---	---	---	---

Reference: Hanai and Hubert (1985)
Column: Hypersil ODS

k' at % acetonitrile

Compound	70	60	50	40	30	20	10
2-Methylpyridine	0.78	0.88	1.11	1.57	2.55	5.05	15.77
3-Methylpyridine	0.86	0.98	1.31	1.84	3.17	6.34	18.92
4-Methylpyridine	0.86	0.99	1.28	1.84	3.04	6.33	19.40
4-Ethylpyridine	1.12	1.40	1.98	3.15	6.04	15.44	---
4-tert-Butylpyridine	1.70	2.41	3.80	7.17	17.95	---	---
2,4-Dimethylpyridine	1.06	1.42	1.92	3.01	5.73	---	---
2,5-Dimethylpyridine	1.03	1.29	1.76	2.75	5.08	12.29	---
2,6-Dimethylpyridine	0.94	1.09	1.49	2.27	4.05	9.30	34.48
Pyrazine	0.36	0.32	0.34	0.38	0.48	0.69	1.29
2-Methylpyrazine	0.44	0.41	0.45	0.53	0.73	1.16	2.75
2,5-Dimethylpyrazine	0.53	0.52	0.60	0.74	1.06	1.94	5.86
2,6-Dimethylpyrazine	0.52	0.52	0.52	0.72	1.03	1.86	5.51
Quinoline	0.93	1.14	1.64	2.68	5.40	14.58	---
8-Hydroxyquinoline	---	---	---	---	---	---	---
2-Methylquinoline	1.07	1.40	2.09	3.65	8.02	24.26	---
4-Methylquinoline	1.26	1.63	2.47	4.39	9.94	24.36	---
8-Methylquinoline	1.29	1.75	2.73	4.92	11.17	32.10	---
5-Aminoindan	0.95	1.31	2.11	3.86	8.46	22.16	---
1-Aminoindan	---	---	---	---	---	---	---
5-Aminoindole	0.36	0.38	0.54	0.78	1.26	2.32	5.34
1-Aminonaphthalene	0.86	1.24	2.09	3.99	9.06	24.48	---
2-Aminonaphthalene	0.85	1.21	2.05	3.98	9.36	26.14	---
1-Aminoanthracene	1.54	2.53	5.05	15.52	42.18	---	---
1-Aminopyrene	2.01	3.41	7.11	18.96	---	---	---

Methylene Selectivity Data

Reference: This Work
Column: Ultrasphere ODS
Mobile Phase: methanol/water

%MeOH	$\log \alpha_{CH2}$	$E_T(30)$ interpolated
48.9	0.2993	58.40
60	0.4011	57.59
70.7	0.5157	56.80
75	0.5725	56.67
80	0.6410	56.41

Reference: This Work
Column: Ultrasphere ODS
Mobile Phase: acetonitrile/water

%ACN	$\log \alpha_{CH2}$	$E_T(30)$ interpolated
32	0.3348	58.81
35	0.3126	58.06
46	0.2422	56.99
50	0.2311	56.80
63	0.1867	55.89
68	0.1678	55.68

Reference: This Work
Column: Hamilton PRP-1

%ACN	$\log \alpha_{CH2}$
0	0.8442
10	0.5679
20	0.4539
30	0.3643
40	0.2983
50	0.2268
60	0.1823
70	0.1518
80	0.1191
90	0.1029
100	0.0754

%MeOH	$\log \alpha_{CH2}$
0	0.8250
10	0.7812
20	0.6916
30	0.6167
40	0.5394
50	0.4610
60	0.3824
70	0.3127
80	0.2241
90	0.1538
100	0.0850

Reference: Karger et al. (1976)
Column: Ultrasphere ODS

%ACN	$\log \alpha_{CH2}$	$E_T(30)$ interpolated*
10	0.5046	
20	0.4299	
30	0.3177	
40	0.2504	
50	0.2056	
60	---	
70	---	
80	0.1159	

%MeOH		
0	0.5415	
5.2	0.5223	62.60
7.5	0.5185	63.37
12.7	0.5031	61.85
21.5	0.4685	60.97
29.7	0.4225	60.16
38.3	0.3495	59.35
49.5	0.2957	58.36
54.5	0.2804	57.94
64.9	0.2228	57.18
75.1	0.1651	56.56
88.2	0.1152	55.98
100	0.0691	55.73

*Interpolated for compositions not measured by using a 3rd degree polynomial fit of $E_T(30)$ versus percent organic modifier.

Reference: Petrovic et al. (1985)

%MeOH	log α (alkylbenzenes)	log α (alkanes)
40	0.4702	---
50	0.3989	0.2921
60	0.3189	0.2432
70	0.2504	0.1944
80	0.1924	0.1422
90	0.1444	0.0897
100	0.0773	0.0377

Reference: Hanai and Hubert (1985)

%ACN	log α
20	0.3206
30	0.2689
40	0.2568
50	0.2037
60	0.1627
70	0.1317

Reference: Dufek et al. (1984)

%MeOH	log α
55	0.2597
60	0.2323
65	0.2071
70	0.1792
75	0.1556
80	0.1281
85	0.1018
90	0.0792
95	0.0548
100	0.0373

Reference: Colin et al. (1983)

%MEOH	log α
0	0.0957
10	0.1457
20	0.1929
30	0.2433
40	0.2991
50	0.3600
60	0.4192
70	0.4722
80	0.5180
90	0.5619
100	0.5716

%ACN	log α
0	0.1101
10	0.1354
20	0.1581
30	0.1780
40	0.1989
50	0.2293
60	0.2863
70	0.3658
80	0.4499
90	0.5460
100	0.5716

APPENDIX B
MODIFICATION OF CURVE FITTER PROGRAM
TO ALLOW CALCULATION OF CONFIDENCE INTERVALS

The following lines were added to the program "Curve Fitter" in order to obtain confidence intervals for the linear regression coefficients reported in this dissertation. The additional lines comprise a short subroutine which is entered at line 1465:

    1465 GOSUB 4010

The subroutine is as follows:

```
4010 XB=C/NS
4020 Z3=T/NS
4030 SX=SQR(U-NS*XB*XB))
4040 Z5=SQR(V-(NS*Z3*Z3))
4050 Z4=W-(NS*XB*Z3)
4070 SS=Z5*Z5-(B*B*SX*SX)
4080 SS=SQR(SS/(NS-2))
4100 T5=3:T9=3.8
4200 B9=T9*(SS/SX)
4210 B5=T5*(SS/SX)
4220 A9=T9*SS*SQR((1/NS)+XB*XB)/(SX*SX)
4229 PR#3
4230 A5=T5*SS*SQR((1/NS)+(XB*XB)/(SX*SX))
4240 PRINT"SLOPE=";B;" +- ";B9;" / ";A5
4250 PRINT"Y-INT=";A;" +- ";A9;" / ";A5
4260 RETURN
```

The user must change line 4100, which contains the t-statistic values for the 90% (variable T5) and 95% (variable T4) levels of confidence.

APPENDIX C
MODIFICATION OF CURVE FITTER PROGRAM TO
INTERPOLATE SPECTRAL PEAK POSITIONS

By adding only a few lines to the program "Curve Fitter," it is possible to automatically calculate the interpolated position of the spectral peak of interest. These additional lines were written with the assumption that the absorbance data (absorbance versus wavelength) have been fit with a three degree polynomial.

The first additional line follows line 1680, where the coefficients were printed out:

1681 A=3*C(4) : B=2*C(3) : C=C(2)

Line 1681 is used to calculate the derivative of the polynomial, which yields the three coefficients, A, B, and C. Next, the quadratic equation is solved for these three coefficients; this is equivalent to setting the derivative equal to zero and solving for the roots:

1682 X1= (-B + SQR (B*B - 4*A*C))/2*A

1683 X2= (-B - SQR (B*B - 4*A*C))/2*A

Finally, the two roots are printed out:

1684 PRINT: PRINT: PRINT "MAXIMUM IS AT ";X1;" OR ";X2;" : PRINT

APPENDIX D
SOLVATOCHROMIC SOLVENT POLARITY MEASUREMENTS

$E_T(30)$ polarity of binary and ternary mixtures of water/methanol/acetonitrile.

H_2O/MeOH/ACN	$E_T(30)$ POLARITY (kcal/mole)
0/100/0	55.63
0/90/10	53.20
0/20/80	54.33
0/30/70	54.88
0/40/60	55.26
0/50/50	55.57
0/60/40	55.62
0/70/30	55.65
0/80/20	55.84
0/90/10	55.75
10/0/90	53.80
10/10/80	54.73
10/20/70	55.00
10/30/60	55.49
10/40/50	55.69
10/50/40	55.84
10/60/30	55.82
80/20/0	60.94
90/0/10	61.43
90/10/0	62.15
80/10/0	60.34
10/70/20	55.95
10/80/10	55.89
10/90/0	55.89
20/0/80	55.09
20/10/70	55.41
20/20/60	55.71
20/30/50	55.99
20/40/40	56.10
20/50/30	56.24
20/60/20	56.32
20/70/10	56.50
20/80/0	56.37
30/0/70	55.71

H$_2$O/MeOH/ACN	E$_T$(30) POLARITY (kcal/mole)
30/10/60	56.02
30/20/50	56.16
30/30/40	56.46
30/60/10	56.93
30/40/30	56.64
30/70/0	56.84
30/50/20	56.75
40/0/60	56.19
40/10/50	56.48
40/20/40	56.80
40/30/30	56.91
40/40/20	57.32
40/50/10	57.41
40/60/0	57.46
50/0/50	56.82
50/10/40	57.25
50/20/30	57.41
50/30/20	57.90
50/40/10	57.97
50/50/0	58.30
60/0/40	57.46
60/10/30	58.04
60/20/20	58.21
60/30/10	58.59
60/40/0	59.17
70/0/30	58.44
70/10/20	58.85
70/20/10	59.44
70/30/0	59.78
80/0/20	59.81
100/0/0	63.12
0/0/100	45.97

E$_T$(30) polarity of binary tetrahydrofuran/water mixtures.

%THF	E$_T$(30) POLARITY (kcal/mole)
10	60.97
15	59.72
20	58.54
30	55.54
40	53.83
50	52.62
60	51.64
70	50.86
80	49.61
90	47.91
95	45.89
100	39.14

π^* , α, and β values for methanol/water and acetonitrile/water mixtures.

%MeOH	π^*	α	β
0	1.081	1.067	0.470
10	1.085	1.010	0.454
20	1.047	0.969	0.500
30	1.028	0.948	0.521
40	0.999	0.903	0.521
50	0.948	0.891	0.549
60	0.900	0.877	0.570
70	0.834	0.889	0.598
80	0.756	0.917	0.626
90	0.667	0.953	0.647
100	0.566	1.015	0.852

%ACN	π^*	α	β
0	1.081	1.067	0.470
10	1.066	0.985	0.464
20	1.035	0.914	0.474
30	0.984	0.873	0.498
40	0.932	0.855	0.533
50	0.876	0.858	0.563
60	0.837	0.850	0.572
70	0.834	0.826	0.556
80	0.771	0.835	0.583
90	0.727	0.794	0.566
100	0.665	0.398	0.370

H_2O/MeOH/ACN	π^*	α	β
50/10/40	0.908	0.860	0.540
50/20/30	0.924	0.858	0.536
50/30/20	0.938	0.885	0.543
50/40/10	0.950	0.871	0.534
60/10/30	0.955	0.871	0.527
60/20/20	0.974	0.867	0.514
60/30/10	0.968	0.724	0.530
70/10/20	0.980	0.899	0.523
70/20/10	1.009	0.912	0.503
90/10/10	1.041	0.940	0.483
33.3/33.3/33.4	0.857	---	---

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211

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BIOGRAPHICAL SKETCH

Bruce Philip Johnson was born in Detroit, Michigan, on February 21, 1958 (at 8:37 AM, New Grace Hospital). He attended public schools in Dearborn, Michigan, through the eleventh grade, at which time his father retired (1975), and his family moved to Tustin, Michigan. He graduated from Pine River High School (Leroy, MI) in June, 1976 and entered Central Michigan University (Mount Pleasant, MI) in August of that year. While attending CMU he worked part-time as a co-op student at the Dow Chemical Company (Midland, MI). In December of 1980 he received a B.S. degree in chemistry from CMU and entered graduate school at the University of Florida in January, 1981. He was awarded a three-year graduate fellowship from the Eastman Kodak Company in May, 1982. He was married to his wife, Bonnie, in December, 1982. Their son, Garrett Chase, was born on April 22, 1986 (1:08 PM EST; The Birth Center, Gainesville, FL). Upon completion of the requirements of the degree of Doctor of Philosophy (August, 1986), he accepted a position with the Eastman Chemicals Division of the Eastman Kodak Company (Kingsport, TN).

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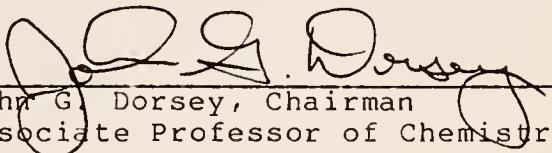
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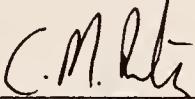
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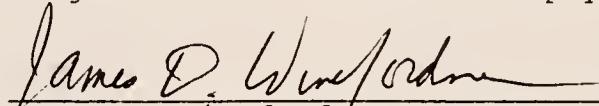
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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1986

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